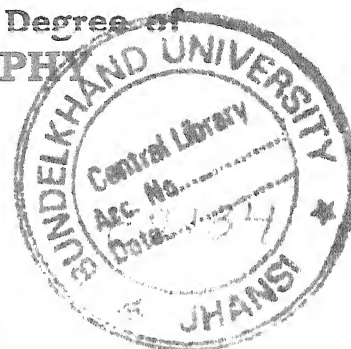


HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF THE OLFACTORY MUCOSA OF SOME FISHES LIVING IN DIVERSE HABITATS



THESIS
Submitted for the award of the Degree of
DOCTOR OF PHILOSOPHY
IN
ZOOLOGY



Under the Supervision
of

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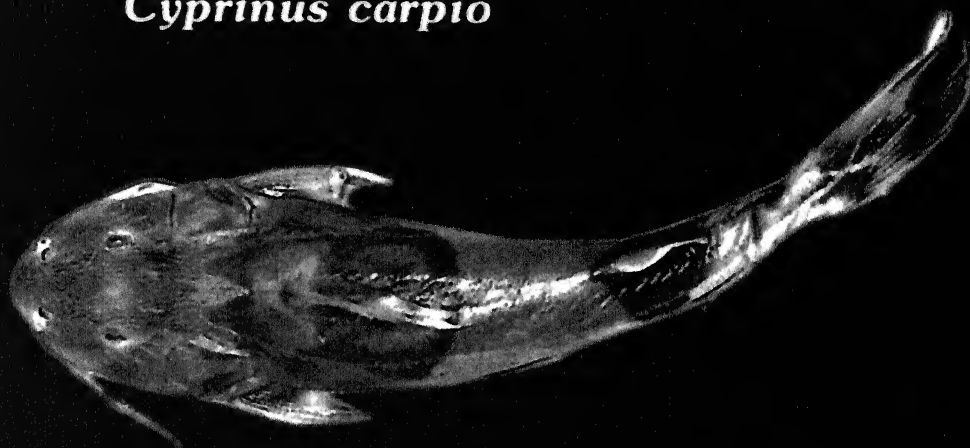


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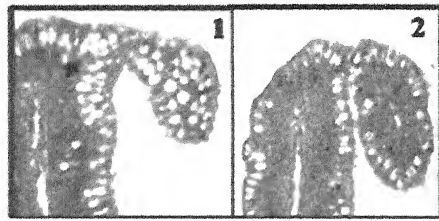
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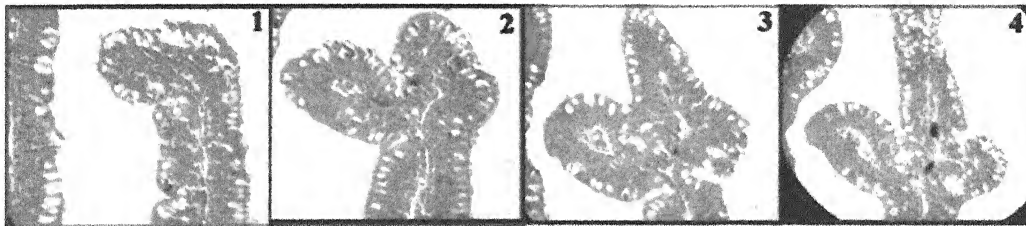
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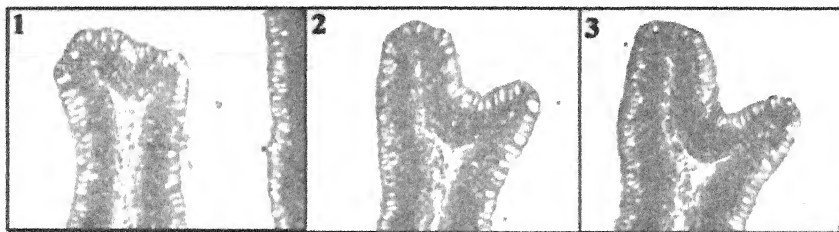
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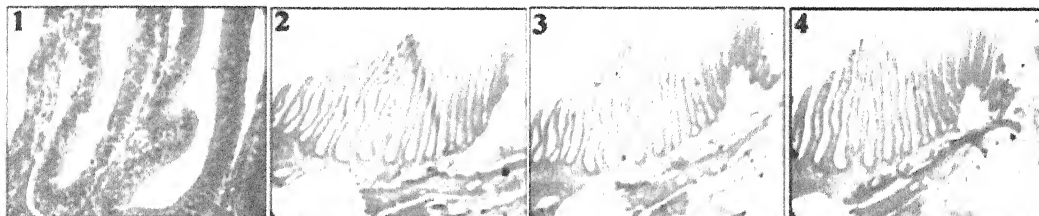
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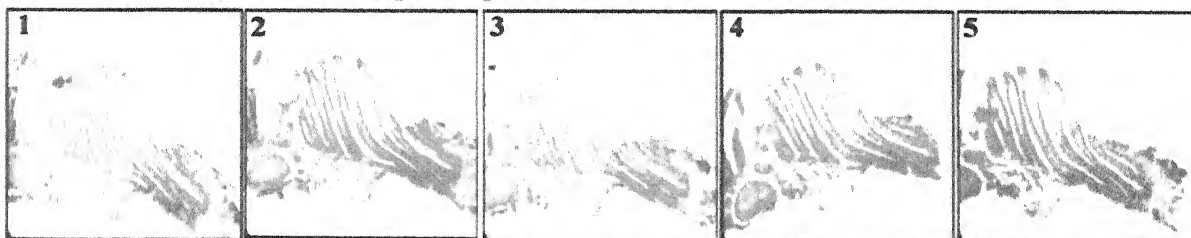
Trifurcation series



Bifurcation series



Stages depicting emergence of minor lamella from mother lamella by the process of outpushing of submucosa and mucosa of mother lamella.



Lateral bud showing detachment from the mother lamella and gradually elongating to join distal end of recipient lamella to enhance the growth of later.



The distal end of lamella discharging "cell ball " by gradual constriction of underlying region. It later joins to the subsequent lamella and contributes lamellar contents to the recipient lamella.

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Certificate

This is to certify that the thesis entitled "**Hitological and Histochemical Studies of the olfactory mucosa of some fishes living in diverse habitats**" embodies the original research work of **Mr. Vijay Kumar Yadav**.

The candidate had worked under my guidance and supervision for the period required under the provision of ordinance 7.

The candidate has put the required attendance during the research period.

Dated : 6th March 07

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ABBREVIATIONS

1	ANT.NAS.OP	Anterior nasal opening
2	ARE.	Areolae
3	BC.	Basal cell
4	BC.Z.	Basal zone
5	BCP.	Blood capillaries
6	BIF.	Bifurcation
7	BM.	Basal membrane
8	C.BALL.	Cell ball
9	CONN. TIS.FIB.	Connective tissue fibres
10	CONS.	Constriction
11	CRY.	Crypts
12	CUN.	Cuneiform
13	CUR. LAM	Curved lamella
14	DEP.	Depression
15	DET.C.BALL	Detached cell ball
16	DIS. LAM.	Distal lamellae
17	DIS.E. LAM.	Distal end of lamella
18	DN. P.N.	Dendrite of primary neuron
19	DN.R.R.	Dendrite of rod shaped receptor
20	ELE.	Elevation
21	ELO.BUD.	Elongated bud
22	ELS.CONN. TIS.	Elastic connective tissue
23	ETH.ACC. NAS.SC.	Ethmoidal accessory nasal sac

24	FIB.	Fibroblast
25	FIL.	Filiform
26	FOL.OLF.	Folium olfactorium
27	FU.LAM.	Fused lamella
28	FUN.	Fungiform
29	GC.	Goblet cell
30	GC.B.	Goblet cell blast
31	GC.TH.	Goblet cell theca
32	GR.PN.	Group of primary neurons
33	HIL.ELE.	Hillock elevation
34	HIN.LAM	Hinder lamella
35	HIS.	Histocytes
36	INI.LAM.	Initial lamella
37	INT.LAM.SP.	Inter lamellar space
38	LAC.ACC.NAS.SAC.	Lacrymal accessory nasal sac
39	MC.	Mast cell
40	MID.LAM.	Middle lamella
41	MIG.BC.	Migratory basal cell
42	MIG.GC.	Migratory goblet cell
43	MIN.LAM.	Minor lamella
44	MOT.LAM.	Mother lamella
45	MSA.	Mucosa
46	MU.	Mucous
47	NAS. BARBLE	Nasal barble
48	NER.SUP.	Nervous supply
49	NMN.FIB.	Non medullated nerve fibre

50	NU.BC.	Nucleus of basal cell
51	NU.PN.	Nucleus of Primary neuron
52	NU.RR.	Nucleus of rod shaped receptor
53	NU.SC.	Nucleus of supporting cell
54	OCI.	Olfactory cilia
55	PIG.	Pigment cell
56	PN.	Primary neuron
57	POST.E.	Posterior end
58	POST.LAM.	posterior lamella
59	POST.NAS. OP.	Posterior nasal opening
60	PRO.E.LAM.	Proximal end of lamella
61	REC.LAM.	Recipient lamella
62	ROS.	Rosette
63	RPH.	Raphe
64	RR.	Rod shaped receptor
65	SAC.SP.	Sac space
66	SC.	Supporting cell
67	SC.Z.	Supporting zone
68	SMSA.	Sub mucosa
69	SR.	Spindle shaped receptor
70	TER.BUD.	Terminal bud
71	TRI.	Trifurcation
72	UN.CRY.	Unexposed crypt
73	VAC.	Vacuole
74	VEN.LAT.ACC.NAS.SAC	Ventro lateral accessory nasal sa

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Chapter-1

Introduction and Historical Review

Introduction and Historical Review

India is the second most populous country of the world with over one billion people. If the current growth rate continue, it will have 1.63 billion people by 2050 and will become the most populous country surpassing china. To fulfill the nutritional requirement of such a vast country, it is necessary to explore other means of food supplements to compromise with grain production. The fishery sector possesses non fish and fish organisms, which play an important role to compensate food requirement in view of rich protein, minerals and other contents required for good human health.

Keeping this in mind, we have choosen this topic to justify the adaptability of fishes in diverse habit and habitat with regard to its olfactory perceptive reactionary characteristics. This provides an opportunity to living organisms to thrive best in diverse ecological conditions and play decisive role in feeding, defence, schooling, spawning, mating and migratory behaviour.

The fishes, therefore, need receptors which can identify the nature of the stimulus to avoid harmful effects. Among the receptors of fish maintaining the contact of the organism with the external environment and responding to the behavioural responses, one of the important senses is olfaction.

Olfaction in fish has a number of special features the essential ones are extremly high sensitivity, ensuring perception of traces of stimulus and slow adaptation. The sense of olfaction is a long range type of reception in which informations are gathered from a distance.

The method of conditioned reflexes made it possible to establish the limits of olfaction in some fishes with a whole series of smells. Teichmann (1957, 1959) demonstrated that trout could perceive phenyl ethyl alcohol at a concentration of 9.9×10^{-9} M. He also noted that the perception power varies markedly from species to species. Liang *et al.* (1998); Liao and Chang (2003); has reported the role of sense organs in the feeding behaviour of Chinese perch and juvenile red drum respectively. Hara (2006) has demonstrated that the feeding behaviour in some teleosts is triggered by single amino acids primarily through olfaction.

Fragmentary accounts on olfactory organ of fishes are available from the second half of the nineteenth century. Most of the earlier workers have given a general account of morphology and anatomy of the olfactory organ. Sophie Pereyaslawzeff (1876) is probably the first to report the anatomy of two fishes *Solea impar* and *Lophus piscatorius*. Important contribution in the field of anatomy of the olfactory organ of fishes are those of Burne (1909) Allisson (1953), Hagelin and Johnels (1955), Kleerekoper and Erkel (1960), Trujillo - Cenoz (1961), Johnson and Brown (1962), Kubiak (1962), Branson (1963), Gooding (1963), Pfeiffer (1963, 1964), Bannister (1965), Moulton and Beidler (1967), Kapoor and Ojha (1972 a, b, 1973 a,b), Hara (1975), Kapoor and Ojha (1971, 1972, 1973a,b, and 1974), Rahmani and Khan (1977, 1980, 1981), Rizvi (1981), Sharma *et al.* (1981), Rao and Finger (1984), Chen and Arratia (1998), Eastman and Lannoo (1998, 2003) and Lannoo and Eastman (2005).

Reviewing the existing literature it is found that the work on the olfactory organ of the European fishes has been carried out to some extent but little work has been done on Indian teleosts. Therefore, keeping this fact in mind we have chosen this topic "Histological and Histochemical studies of the olfactory mucosa of some fishes living in diverse habitat", so as to expose its histology, histochemistry and other behavioural aspects of fishes selected from diverse habit and habitat. This study will also focus on fish adaptability in diverse habit and habitat with regard to its olfactory perceptive reactionary characteristics. This will allow us to establish and cultivate fishes in diverse ecological conditions and to attain better productivity in Natural and Man made ecosystem.

With regards to the study of histochemistry, our knowledge is mainly based on the study of mammalian olfactory mucosa. Important contributors in this field are Bourne (1948); Baradi and Bourne (1951, 1953); Burckhardt and Ehrmantrout (1955); Amicis and Zarzoli (1957); Bronshtein (1960, 1962 a, b, 1965); Jinin (1965); Duveau and Gerebtzoff (1967); Shantha and Nakajima (1970); Belanger *et al.* (2003); Cinar and Senol (2006); Merchetti *et al.* (2006); Besides this, practically nothing is known about the pattern of distribution of contents in different cellular elements of olfactory mucosa.

Therefore, in the presents research work, the attempt has been made to localize the histochemical contents of the olfactory epithelium in relation to demonstration of acid and alkaline phosphates, lipids, phospholipids, glycogen, acid mucopolysaccharides, and meta chromasia in *C. carpio* and *T. mossambica*.

For the present histological work the author has selected the following three species of fish : *Cyprinus carpio* and *Bagarius bagarius* and *Tilapia mossambica*. The habit, habitat, distribution and identifying character of the fishes are given herein :-

***Cyprinus carpio* (Linnaeus) :**

Cyprinus carpio var communis (German strain) Linnaeus, is commonly known as "mirror carp" and originally native to the region from Black and Caspian seas to Turkistan. From there it was spread by introduction throughout most of the temperate waters of the world.

According to Jhingran (1975) *C. carpio* is an exotic fish but now it is commonly cultivated singly as well as along with major Indian carps. He further described that "mirror carp" was brought in 1936 from Ceylon to Nilgris and stocked in Ootacamund lake. It was generally thriving best at high altitudes, but for the first time, on April 18, 1955, it was introduced in the pucca tank at Nahan (Sirmur district, Himachal Pradesh). At present this common carp enjoys global distribution, occurring in tropical as well as temperate regions and acclimatized to a variety of habitat and extremes of environment (Ali Kunhi, 1966).

The body of *C. carpio* var cummunis (German Strain) is moderately deep; slightly compressed and fully covered by regularly arranged rows of scale; Mouth is directed forward and protrucible and two pairs of barbels are present on upper lip.

C. carpio is an omnivorous feeder and its natural food constitute small animals and parts of the plants. It feeds voraciously by rapidly protruding and retracting the jaws. The fish is very much active in day

and actively swims near the surface of water. It very easily becomes pet to the master and usually comes to bank in shoals.

***Tilapia mossambica* :**

It belongs to family Cichlidae which includes perch like fishes with oblong, compressed body and covered by moderate size ctenoid scales upto head. It is found in both fresh and brakish water.

This is an East African species that has been widely introduced and naturalized in India. It was first introduced in India in 1952 by Central Marine Fisheries Research Station, Mandapam. It is a medium sized cichlid, readily identified by its blackish blue colouration (males) and its fins are beautifully bordered with red. Its large head and wide mouth are further pointers. The dorsal and anal fins have prolonged and pointed tips and the tail is rounded. It prefers ponds, lakes, streams, rivers and estuaries. It is often found in flood waters and ditches.

The species is an excellent subject for studying territorial behaviour in fish, both in the wild and in captivity. Due to their aggressive nature and omnivorous diet, Tilapias out compete most other native species and thus tend to dominate water bodies wherever they occur.

This fish has a characteristic mode of parental care. The females carry the eggs and young ones in the mouth and is popularly called mouth breeding cichlid.

It is a fast growing hardy fish but does not survive at a temperature lower than 10°C. It can tolerate wide range of salinity and therefore recommended for culture in coastal regions. When cultured in sewage water mixed with fresh water it is reported to yield 7000

Kg/ha/yr, in Indonesia. It can also be successfully cultured in rice fields. *Tilapia mossambica* also serve as a forage fish and when cultured with *Channa striatus* in ponds, the yield is considerably increased as it is utilized as food by the murrels.

***Bagarius bagarius* (Hamilton) :**

It belongs to family Sisoridae. It is a primitive fish, formerly widely distributed in south-west asia. It is also called fresh water shark because of its ferocity and under hung mouth.

Head broad and flattened; shoulders raised with body tapering behind to a slender tail stem. The caudal fin is deeply forked. Its colour is variable, grey or yellowish brown with large, irregular, dark brown or sometimes black markings and crossbands. The fins are usually black based and also banded.

It inhabits in rapids, rocky pools, as well as sandy or muddy reaches where ample shelter is provided within the deeper broken up and silty substratum. It has also been called a sluggish form, occupying the bottom of deeper portions of the rivers.

It is a good sport fish but requires strong tackle; live bait, particularly bham is best for catching this fish. It regularly forms major bulk of the catches by netting and lining operations. Sometimes it is caught by spearing also, particularly during the monsoons when large sized individuals are seen trapped in inundated nullahs. It is a monsoon breeder (July-Sep).

For a long time the independent existence of olfaction in fishes was disputed. Nagel (1894) and a number of authors refuted the existance of true olfactory sense in aquatic animals, premising that

the organ of smell can be stimulated only by gaseous substances. They were of the view that chemical stimulation in aquatic animals could only be mediated by taste.

Since then, many investigators contributed to the study of this problem of physiological differences between the senses of smell and taste in fish. Important among them are Von Uexcull (1895); Herrick (1908); Parker (1910, 1913); Sheldon (1911, 1912); Copeland (1912); Strieck (1924). The most convincing evidence of the olfaction in fish independent of taste was obtained by Strieck (1924). He trained minnows, *Phoxinus phoxinus* to discriminate pure odorous (coumarin, skatole and muscone) and taste (glucose, acetic acid and quinine) substances. These substances were readily detected by intact minnows. However, trained fish were unable to discriminate odorous substances after the forebrain was removed, although they could perceive taste substances. The correctness of Strieck's findings was subsequently confirmed experimentally by several authors who studied olfaction and gustatory sensitivity in fish (Frisch, 1941, Hasler, 1954 and others).

Blaue (1884) published a paper titled "The olfactory membrane in fishes and amphibia", in which he discussed the general anatomy of olfactory pit and rosette in *Belone*, *Exocoetus*, *Trigla*, *Esox*, *Umbra*, *Cottus*, *Gobius* and *Gadus*.

Bateson (1889) studied the various sense organ in fishes and laid a special stress on the study of the olfactory organ of fishes. In a very generalised manner, he pointed out the main type of the structure of rosette; elongated in eels, oval in the majority of fishes or

circular in *Cottus* and exceptional type in which the leaflets are arranged in parallel series in a single row (*Pleuronectus* and *Hippoglossus*). He also described the arrangement of plates in the olfactory rosette in the following manner : (i) In skate and dog fish plates are arranged in a radiating manner on the inside of a hollow capsule, like the septa of orange (ii) The Conger and eel have the plates arranged in two rows on each side of the central raphe, upon which the two rows are folded longitudinally so as to form the lining of the olfactory tube (iii) The olfactory organ are provided with the plates which are fitted together in radiating manner forming a convex eminence in the olfactory chamber. The whole organ is either circular as in *Cottus* and *Motella mustela* or elliptical as in mackerals (iv) In *Pleuronectus* and *Hippoglossus Vulgaris* only one row of the olfactory plates is present and the plates thus arranged in a single series lie in a direction parallel to the long axis of the body.

Bateson (1889) on the basis of the olfactory behaviour divided the fishes into two categories (i) group of fishes which hunt their food with the help of vision and no reaction to the smell of food was observed (ii) second group of fishes which seek their food by the smell and vision was never used for this purpose. He concluded that all the fishes hunting by smell are to some extent nocturnal animals. His conclusions were based on the study of following group of fishes eel (*Anguilla anguilla*), marine barbot (*Gaidropsorus tricirritus* and *G. mustela*), common sole (*Solea vulgaris*), dog fish (*Scyliorhinus canicula*), African long fish (*Prostopterus annetens*), Ray (*Raja batis*) and Starlet (*Acipensor ruthenus*).

Solger (1894) presented the idea of water circulation through the olfactory chamber. According to him, the alternate compression and expansion of the accessory sac synchronously with the respiratory movement cause the water to flow through the olfactory cavity. The same conclusion was latter drawn by Johnson and Brown (1962) in black rockfish, *Sebastes melanops*. Pippings research (1926) showed that the olfactory capacity of fishes is in close relation to the nature of the movement of water through the olfactory sacs. On the basis of this relationship Malyukina *et al.* (1969) divided fish into four groups :

- (i) The first group includes those in which the flow of water passes through the olfactory sacs; water enters and comes out through the olfactory sacs only at the time of forward movement.
- (ii) In the fish of second group the movement of water is caused by the activity of accessory sacs; water enters and comes out through both nostrils i.e. the unidirectional flow of water is absent.
- (iii) In fish of the third group the mechanism of the water movement characteristic of fish of the second group is supplemented with the unidirectional movement of water at the time of respiratory passes, which are caused by the activity of the ciliated epithelium.
- (iv) In the olfactory sacs of the fourth group the flow of water takes place constantly from the anterior side in a backward direction. In this process the respiratory movement and the activity of cilia take part.

Experiments have shown that the fish belonging to the fourth group have highly developed olfaction which plays a significant or even decisive role in the location of food. In fish of the first and second group, olfaction is weakly developed and does not contribute to the location of food.

Because of large differences in olfactory ability in different species, Frisch (1941) proposed the same classification into microsmates and macrosmates, that has been accepted for animals of other classes.

According to Doving *et al.* (1977) and Doving and Thommeson (1977) the water circulation through the olfactory chamber is technically denominated as Isomates and Cyclomates types. In the former group ciliation of olfactory epithelium is responsible for the water circulation through the olfactory chamber, whereas in later group compression and expansion of the accessory sacs in relation to skull bone, bring about the transportation of water through the olfactory epithelium.

Devivot and Gobet (1979) substituted the term Heterocyclomates for Isomates and Autocyclomates for Cyclomates. In the former group, fishes are dependent on respiratory movement for the circulation of water through the olfactory chamber, whereas in the later group ciliary action is solely responsible for creating the water current through the olfactory chamber.

Rahmani (1979) in addition to Doving *et al.* (1977), further elaborated the classification of fishes with regard to the circulation of water through the olfactory chamber. He put forward another

denomination as Amphisomates, besides isomates and cyclomates. In this group, ciliary movement as well as the pumping activity of sacs brings about water circulation. The remarkable observation is the presence of window in some lamellae of *Colisa fasciatus* which facilitates easy water circulation through the olfactory rosette.

Kyle (1899) studied the presence of accessory nasal sac (a posteriorly lobed diverticulum of the nasal sac proper) in the species Hippoglossus, Pleuronectus, Rhombus, Solea and Cynoglossus. He also concluded that the accessory sacs are the characteristic of semi sedentary fishes.

Burne (1909) described the olfactory organ of teleost fishes and observed that the olfactory chamber belonging to 32 families and 52 genera differ comparatively in shape and size. He distinguished four types of olfactory rosettes and classified them in columns and types of Bateson (1889) :

- (i) First type of rosette is oval in shape and is very commonly observed in the fishes studied by him (Bateson, 1889, type 3; Burne, 1909, column I).
- (ii) Second type of rosette is circular in shape and is found in Cyclopterus, Bovichthya, Cottus, Esox, Orestias. It is provided with lamellae radiating in all directions (Bateson, 1889, type 3 ; Burne, 1909, Column III).
- (iii) Third type of rosette is elongated with their lamellae arranged in parallel series at right angle to it (Bateson, 1889, type 2 ; Burne, 1909, column II). Such type of rosette is found in most of the eels and to a less extent in Siluroids and Soles.

- (iv) Fourth type of rosette is with transverse axis to the internarial line and the lamellae are attached to its posterior border in parallel series (Burne, 1909, column IV). Such rosette are observed in *Ophicephalus*, *Hippoglossus* and *Pleuronectus*.

According to Hara (1975) fish with round rosettes normally have only a few lamellae and usually show little or no behavioural responses to olfactory stimulation (microsmatic). While in species having oval and elongate rosettes (Macrosmatic forms), the sense of olfaction is highly developed.

The olfactory system in fishes can be roughly divided into three parts:

- (i) a peripheral part, represented by the olfactory organ which houses the neuroepithelium and the olfactory bulb;
- (ii) an intermediate part, the anterior olfactory nucleus; and
- (iii) a central part, located mainly in the paleocortical area.

The olfactory organs of fishes are diversely developed. At one extreme they are well developed (Macrosmatic forms) such as in elasmobranchs and most eels, and at the other they are poorly developed (Microsmatic forms) such as in pike, flying fish, stickle back, pipe fish and angler fish.

The olfactory organ in the fishes are represented by a pair of olfactory pits which in sharks and rays are located on the ventral surface and in sturgeon and bony fish on the dorsal surface of the head. Each olfactory organ consists of a cavity lined with the olfactory epithelium, the surface of which is increased because of the formation of serial folds or lamellae. Each nasal pit generally opens outside by

two openings, anterior inlet and posterior outlet, which are separated by a nasal flap or bridge. The patterns of the olfactory flap vary greatly from species to species.

Among the bony fishes the differences in size, structure and extent of development of the olfactory organ are very great. Here significant variations are observed in the number and size of the olfactory openings. The location, form and degree of development of folds in the olfactory rosette of bony fishes varies significantly (Burne, 1909; Leirmann, 1933, Schmalhausen, 1962). The folds significantly increases the surface of the olfactory organ. The number and size of the folds also increases with the age (Schmalhausen, 1962; Pfeiffer, 1963, 1965).

The number of folds in the olfactory sacs of an adult individual and the degree of their development have been taken as an indicator of the olfactory capacity of the fish of the given species. However this correlation most probably does not always hold good. One should also take into account the fact, that, in many fish the olfactory epithelium does not entirely cover the folded surface. The sensory elements are not uniformly distributed over the surface of the folds either. Fish of different species vary considerably in the count of sensory elements per unit surface area of the epithelium, their localization in the rosette and the predominance of some type of sensory element in the olfactory epithelium.

According to Hara (1975), the paired olfactory pits in eels and morays are long and extend from tip of the snout to the eye orbits. Such fishes have acute sense of smell. While in certain puffers

(Tetradontidae), which are highly visually oriented reef fishes, the olfactory pits are totally absent and nasal flaps are exposed to water. Such fishes have regressed capacity of sense of smell. Marshal (1967, 1971) reported that bathypelagic fishes (ceratiod angler fish and cyclothone species) have evolved distinct sexual dimorphism in olfactory organisation and size. In these fishes the males have large olfactory organs while these are small and regressed in females.

The olfactory epithelium which lines the nasal sac is generally raised from the floor of the organ into a complicated series of folds or lamellae to make a rosette. The arrangement, shape and degree of development of the lamellae in the olfactory rosette of telosts vary considerably from species to species. Normally, a central ridge or raphe is formed rostorocaudally on the bottom of the olfactory chamber. From this first ridge a varying number of transverse lamellae radiate. The development of the lamellae begins caudally and progresses rostrally, so that the oldest and largest lamella is located most posteriorly.

Kapoor and Ojha (1973) reported in *Channa punctatus* that the lamellae in the olfactory rosette are arranged with their long axis parallel to the antero posterior axis of the body. They also reported that new lamellae are added at its two lateral ends. Thus the number of lamellae within a species depends on size of the individual.

The variation in the number of lamellae have been reported by various authors in different species: Wunder (1957) reported two in *Gasterosteus aculeatus*; 9-18 in *Esox lucius* ; 11-19 in *Phoxinus phoxinus*; 14-18 in *Salmo gairdneri*; 30-32 in *Lota lota*; 60-90 in

Anguilla anguilla; Shibuya (1960) reported 80-90 lamellae in *Channa argus*; Pfeiffer (1964) reported 230 lamellae in *Haplopagrus guentheri*; Watling and Hillemann (1964) reported 11-15 lamellae in Arctic grayling (*Thymallus arcticus*); Hara, Law and Hobden (1973) found 12-14 lamellae in *phoxinus phoxinus* and 12-16 in *Coregonus clupeaformis*; Lannoo and Eastman (2005) reported 3-4 lamellae in Antarctic eelpouts; Chen and Arratia (1993) found 20-32 lamellae in large acipenserids, 13-18 lamellae in polyodontids, 8-10 lamellae in lepisosteids and *Amia* may have over 100 lamellae. In teleosts, the number of lamellae varies from none or a few to over 200 lamellae.

In addition to the formation of new lamellae, each lamellae increases in size. Teichmann (1954) for the first time reported secondary lamellae in rainbow trout. However, he thought that it might be an artefact caused by fixation. Pfeiffer (1963) latter confirmed the presence of secondary lamellae in pacific salmon, as well as in rainbow trout. Secondary foldings have also been observed by Walting and Hillemann (1964) in grayling; Bertmar (1972) in Baltic sea trout; Sutterlin and Sutterlin (1971) in Atlantic salmon; Devitsyna (1972) in *Lota lota*; Hara *et al.* (1973) in brook trout and lake white fish; Bashor *et al.* (1974) in gar fish. Chen and Arratia (1993) also reported secondary lamellae in acipenseriform, lepisosteids and some teleosts. They also reported tertiary lamellae in *Acipenser oxyrinchus*.

The above authors were of the view, that, the secondary folding process of the primary lamellae results in an increase in the surface area of the olfactory epithelium. However, all or most of the secondary lamellae are devoid of receptor cells. This increase in the surface area

of the olfactory epithelium results in an increase in total olfactory capacity.

Teichmann (1954), Ojha and Kapoor (1972, 1973) and Kapoor and Ojha (1973) made attempts to relate the total area of the olfactory epithelia in different species to their particular olfactory sensitivities. Teichmann (1954) on this basis classified the fishes into three groups—

- (i) Species in which the eye and nose are well developed (Phoxinus and Gobio).
- (ii) Species in which the eye is better developed than the nose (Esox and Gasterosteus).
- (iii) Species in which the nose is well developed, compared with the eye (Anguilla and Lota).

However, there is no simple relation between the surface area of the olfactory epithelium and the number of receptors it contains. In no fish is the sensory epithelium uniformly distributed on the surface of the olfactory lamellae, but it generally occurs in isolated sensory areas. According to Holl (1965), there are three types of arrangements of sensory epithelium in the lamellae :

- (i) Continuous except for the dorsal parts of the lamellae (Ictalurus, Anguilla, Salmo etc).
- (ii) Separated in large areas between the lamellae (ESOX), and ,
- (iii) Dispersed in small islets (Phoxinus, Cyprinus etc)

The microscopical study of the vertebrate olfactory organ began as early as in 1850. However, the olfactory epithelium of fish has not received as much attention as that of other vertebrates. Schultze (1856) and Kleerekoper (1969) described all the cell types and their

fibre connection in the olfactory epithelium. Since then it has been recognised that the olfactory epithelium in vertebrates consists of three cell types : receptor cells, supporting cells and basal cells.

Grimm (1873) also reported, that, the olfactory epithelium in fish as in other vertebrate animals consists of two kinds of cells : Sensory and supporting cells. Sheldon (1912) ; Gasser (1956) and others latter reported that the sensory and supporting cells are lined with a layer of basal cells which is bordered with a basal membrane and supported by connective tissues. Some important references on fine structure of olfactory epithelium in fishes are those of Hopkins (1926) ; Kolmer (1927); Allison (1953) ; Truzillo-Cenoz (1961) ; Branson (1963); Bannister (1965); Wilson and Ivanov (1965); Vinnikov (1966); Wilson and Westernman (1967); Thornhill (1966); Gemne and Doving (1969); Schulte and Holl (1971) ; Schulte (1972); Lawry (1973); Breipohl *et al.* (1973 a,b); Muller and Marc (1984); Moran *et al.* (1992); Byrd and Brunjes (1995); Fishelson (1995):

The supporting cells in most fish have cilia at their distal ends, the movement of which produces the directed current of fluid in the olfactory sac. Holl (1965) reported that in some species (*Anguilla*, *Myxoxcephalus* etc) large flask shaped mucous cells are interspersed among supporting cells. Popova (1966) also observed among the supporting cells, a peculiar type of cells filled with mucous contents of granular polysaccharide nature, which most probably perform a secretory function.

Dogel (1886) reported that, sensory/receptor olfactory cells in fish are found in three forms: filamentous, rod-shaped and cone-

shaped. The peripheral outgrowths of the olfactory cells terminates in pin-like thickenings, carrying one or more hairs of significantly smaller size than the ciliated structures on the supporting cells. The hairs are observed to be in constant motion, which differs significantly from the movement of the cilia. The olfactory hairs according to Vinnikov (1965) are the main elements of perception and their movements are directed in search and location of odorants.

Bannister (1965) in *Phoxinus phoxinus* reported three distinct types of receptor cells : (i) those bearing cilia, (ii) those bearing microvilli, and (iii) those bearing neither cilia nor microvilli, but rising as a simple rod into the mucus. Schulte (1972) also observed such type of morphologically different receptor cell types in the eel. Gemne and Doving (1969) described the qualitative and quantitative aspects of the normal fine morphology of the olfactory receptor cells in *Lota lota*. They discussed the role of the cilia and microvilli in chemoreception.

In addition to the usual cell types, a new type of cellular element, the secondary neuron or spindle shaped cell, has been reported in the olfactory epithelium of *Channa punctatus* by Kapoor and Ojha (1972C) and Ojha and Kapoor (1973) in *Labeo rohita*. Bertmar (1972d, 1973) also found a labyrinth cell, a cell type unique to vertebrates, in the olfactory epithelium of Baltic sea trout. He suggested that these cells probably help to maintain an optimum ion balance, which is of great ecological importance to this migratory species.

Muller and Marc (1984) proposed three distinct morphological classes of receptors in fish olfactory organs: (i) type I ciliar cells, (ii) microvillar cells and (iii) type II ciliar cells. They observed that type I ciliar cells are similar to ciliary olfactory receptors found in all vertebrate classes. Microvillar cells are present in the olfactory organs of most fishes and in the tetrapod vomeronasal organ. Type II ciliar cells have often been described as respiratory type or ciliated nonsensory cells. They are structurally similar to respiratory epithelial cells in the nasal cavities of tetrapods and have motile cilia that beat synchronously, indicative of their role in mediating fluid flow over the olfactory epithelium. In addition to the three receptor types described above, cells resembling receptors with rod like distal processes were observed with scanning and transmission electron microscopy. These "rod cells" are sometimes considered as separate receptor type in fishes.

Zeilinski and Hara (1991 ; 1998) used electron (transmission and scanning) microscopy to examine ultra structural changes in the olfactory epithelium of rainbow trout following unilateral olfactory nerve section. They observed that, both ciliated and microvillar receptor cells degenerate and subsequently differentiate from unidentified precursor cells. They also investigated the morphological and functional differentiation of the olfactory receptor cells in developing rainbow trout (*Salmo gairdneri*) embryos.

Devitsyna (1972) compared two marine species (*Gadus moru* and *Eliginus novaga*) with a fresh water species *Lota lota* on the basis of the histological structure of the olfactory epithelium and bulb. He

observed quantitative distribution of receptor cells along the surface of folds and found that it is characteristic for each species.

Bertmar (1972) described the olfactory organ of trout on the basis of ecological adaptation. He further stressed on the cell population of the olfactory epithelium and defined blastema cells as basal cells which divide into goblet cells, primary receptors and primary supporting cells. He also mentioned fibroblast cells.

Zeiske *et al.* (1976) studied the olfactory epithelium of two cyprinodontidae species by transmission and scanning microscopy. The relatively flat floor of the olfactory organ is covered by sensory and non-sensory epithelia. Non-sensory epithelium separates the distinct area of sensory epithelium. The non-sensory stratified squamous epithelium contains numerous goblet cells and surface cells with microridges. The sensory epithelium bears basal supporting and two types of sensory cells i.e. ciliated and microvilous receptor cells.

Yamamoto and Ueda (1977; 1978 a,b,c,d,e,f) used scanning microscopy to describe the ultra microscopic structures of the olfactory epithelium of the representatives of the orders Salmoniformes, Clupeiformes, Cypriniformes, Gasterosteiformes, Channiformes, Symbranchiformes, Anguilliformes and Myctophiformes. Their main stress was on the different types of ciliation and intercellular contents of the cells of the olfactory epithelium. They described following types of the cells on the basis of their surface specialisation : (i) cells bearing many long cilia on wide and flat surface (type I ciliated cells) ; (ii) those bearing several short cilia which project radially from the round cell apex (type II ciliated

cells); (iii) those bearing no cilia but a tuft of numerous microvilli (microvillus cells); (iv) those bearing neither cilia nor microvilli but protruding as a simple rod from surface (rod cells). Their internal structures are reported to have similar internal microorganelles.

On the basis of surface specialisation in the olfactory epithelium, Yamamoto and Ueda (1978e) reported that fish with dense cilia arising from type I ciliated cells are believed to have predominantly developed olfactory sensitivity such as eels (Schulte, 1972; Yamamoto and Ueda, 1978c), Salmon (Bertmar, 1972; Yamamoto and Ueda, 1977) and Cod (Lowe and MacLeod, 1975). Contrary to this, fishes are having less developed olfactory sensitivity where epithelium lacks type I ciliated cells and cilia are dispersed into small islets such as in Atheriniformes (Zeiske *et al.*, 1976), Stickle backs (Bannister, 1965; Yamamoto and Ueda, 1978d).

Pandey and Mishra (1980) studied olfactory apparatus and reported that in *Cirrhinus mrigala* and *Labeo rohita* lamellae are double layered and bears central connective tissues, consisting of usual cell types in the form of receptor, supporting, basal and goblet cells. They have tried to classify olfactory rosette of *C. mrigala* and *L. rohita* according to Bateson (1889) and Burne (1909). They have also described these fishes as eye-nose fishes according to Teichmann (1954).

Sharma (1981) reported that the olfactory epithelium of lamellae exhibits cellular activities like budding, detachment, cellular extrusion, curving and the migration of mucous secretory goblet cells in different fishes in his research work.

Singh and Singh (1986) carried out their investigation on the olfactory organ of four hill stream fishes. They reported that the olfactory epithelium is composed of ciliated cells, microvillus cells, supporting cells and pigment granules. Rod cells were found only in the lamellae of *Schizothorex richardsonae*. Apertures or holes and tufts of microvillus cells were also observed in the olfactory lamellae of *Puntius chilinoides*.

Sinha (1986) described the functional anatomy of the olfactory organ of *Sicamugil cascasia* (HAM) in which two accessory nasal sacs are situated dorsally and ventrally to the main olfactory chamber. The author studied the working of the accessory nasal sacs and its role in effecting replacement of water in the olfactory chamber of the fish.

Waghray (1986) reported sexual dimorphism in electric ray on the basis of shape and size of the olfactory organ. He reported that the olfactory organ is kidney shaped and more rounded in male while slightly elongated and narrow in female.

Kashiwayanagi *et al.* (1987) reported the changes in membrane potential and membrane fluidity in response to various odorants in a suspension of porcine olfactory mucosa.

Doroshenko and Motavkin (1987) observed variations in number and arrangement of the olfactory rosette folds, as well as in olfactory epithelium, which they named as receptory and indifferent epithelium. They further pointed out that the olfactory epithelium varies greatly interspecifically in the arrangement of receptor and secretory cells. The olfactory epithelium contains three major cell types, which are

easily identified on the basis of location of nuclei, which distinguishes them from cytoplasmic zone. The cell types identified are :

- (i) Sustentacular cells : In them the nuclei is located in the most superficial part of the nuclear zone. These cells extends through the entire thickness of the epithelium and does not contain cilia.
- (ii) Basal cells : In these cells the nuclei is situated in the deepest part of the nuclear zone, immediately adjacent to the connective tissues and have only a small amount of cytoplasm, which is confined to vicinity of the nucleus and does not reaches the surface. In this zone mainly, there is a supply of lymphoid wandering cells and macrophages, some of which move into other zones and phagocytize the dead or degenerating cells.
- (iii) Receptor cells : In them the nuclei is located in the broadest part of the nuclear zone. These cells contain a distal process that extends from the perikaryon to the surface where they possess a bulbous expansion called the olfactory vesicle. The proximal part of the receptor cells extends towards the basal region of the olfactory layer, where it continues as a slender axon and along with the axon of other receptor cells.

Yadav (1988) studied the histomorphology of the olfactory organ of fresh water fishes. He reported that the variation in the cellular composition of the lamellae, not only occurs in different fishes but also in the lamellae of the same rosette of an individual fish. The author also reported deepenings and elevations in the olfactory epithelium and concluded that the former one is in the form of crypts,

which are richly supplied with primary neurons and takes the shape of "Olfactory bud".

Dubey (1991) after doing comparative histological study of olfactory epithelium of *Colisa fasciata*, *Clarias batrachus* and *Chandana nama* concluded that lamellae is provided with a number of microformations in the form of cell ball, curving, mucosal inpushings and number of structural variations.

Moran *et al.* (1991) described the ultrastructural neurobiology of the olfactory mucosa of the brown trout, *Salmo trutta*. They found that the trout olfactory contains five cell types : Ciliated epithelial cells, ciliated olfactory receptor cells, microvillar olfactory receptor cells, supporting cells and basal cells. The ciliated and microvillar receptor cells are primary sensory bipolar neurons whose dendrites make contact with the environment and their axons travel directly to the brain. Therefore, the substances can be transported directly from the environment into the brain via these naked neurons. Since fish cannot escape from the water in which they swim, and since that water may occasionally contain brain-toxic substances, the ability to close off and later reopen this anatomic gateway to the brain would confer a tremendous selective advantage upon animals that evolved the brain sparing capacity to do so. They also observed that, when the olfactory nerve is cut, both ciliated and microvillar olfactory receptor cells degenerate within 2 days and are morphologically intact again within 8 days. They also observed that when wild trout are taken from their native stream and placed in tanks with elevated copper concentrations, ciliated and microvillar cells degenerates.

Replacement of these trout into their stream of origin is followed by morphologic restoration of both types of olfactory receptor cells.

Zielinski and Hara (1991) examined the ultra structural changes in the olfactory epithelium of rainbow trout (*Salmo gairdneri*) following unilateral olfactory nerve section, by the use of scanning and transmission electron microscopy. They observed that both the ciliated and microvillar receptor cells degenerated and subsequently differentiated from unidentified precursor cells.

Hansen and Zeiske (1993) investigated the development of the olfactory organ in the zebra fish, *Brachydanio rerio*. They observed, that the olfactory placode is formed by a subepidermal layer of cells. These cells differ from the brain or the epidermis cells and they do not mingle either with epidermal or with brain cells. No migration of cells from the brain or the epidermis towards the subepidermal cells layer has been observed. The cells of the subepidermal layer seem to form all cell types of the olfactory mucosa i.e. basal cells, ciliated and microvillous receptor cells, supporting cells and ciliated non-sensory cells. They also observed that the axons grow into the forebrain at a very early stage when the epidermis still covers the placode completely. Dendrites grow out when the epidermis separates, building the olfactory pit. This process implicates neither cell-lysis nor cell degeneration. The olfactory pit forms a rosette with a midline raphe and olfactory lamellae.

Getchell and Getchell (1991) investigated the fine structural aspects of secretion and extrinsic innervation in the olfactory mucosa of vertebrates. They described the ultrastructure of olfactory mucous

and of the secretory cells that synthesize and secrete olfactory mucous in the vertebrate olfactory mucosa. Bowman's glands are present in the olfactory mucosa of all vertebrates except fish. They consist of acini, which may contain mucous or serous cells or both, and ducts that traverse the olfactory epithelium to deliver secretions to the epithelial surface. Sustentacular cells are present in the olfactory epithelium of all vertebrates. In fish, amphibia, reptiles and birds, they are secretory while in mammals they are generally considered to be non-secretory. Goblet cells occur in the olfactory epithelium of fish and secrete a mucous product.

Chen and Arratia (1994) studied the olfactory organ of acipenseriformes and compared it with other actinopterygians. The position and structure of the olfactory organ and its openings vary among actinopterygians. The anterior nasal opening is a simple perforation in the skin of many extant actinopterygians (eg. acipenseriformes, lepisosteids and primitive recent teleosts) and represents the primitive condition. Polypterids and *Amia* each exhibit a derived condition, in which the anterior nasal opening extends into a tube. The olfactory organ is relatively far away from the anterior end of the elongate rostrum in acipenseriformes, whereas the olfactory organs are closer to the anterior end of the snout in extant actinopterygians (eg. polypterids, lepisosteids and amiids). In adults, olfactory organs are cuplike structures in most actinopterygians, but these organs are tube like in polypterids. Among extant actinopterygians, a nasal diverticulum is present only in polypterids.

Teleosts have accessory nasal sacs, but chondrosteans, polypterids, lepisosteids and amiids lack them.

They also described that the olfactory rosette is formed by primary folds or lamellae that may be placed anterior, lateral, posterior, and / or medial to the axis of the organ. Large acipenserids have 20-32 lamellae, polyodontids have 13-18 lamellae, lepisosteids have 8-10 lamellae and *Amia* may have over 100. In teleosts, the number of lamellae varies from none or a few to over 200. Secondary lamellae are present in acipenseriforms, lepisosteids, and some advanced teleosts. Tertiary lamellae are present in *Acipenser oxyrinchus*. The arrangement of the primary lamellae in relation to the axis of the organ results in at least 11 patterns of the olfactory rosette in actinopterygians. Lamellae that are enclosed in a tube like sac and that have an anteromedial diverticulum are specializations of polypterids. Primary lamellae anterior, lateral and posterior to an elongate axis are characteristic of lepisosteids. The presence of primary lamellae lateral, medial and posterior to an elongate olfactory axis is a synapomorphy of Halecomorpha (*Amia* plus teleosts).

Byrd and Brunjes (1995) used a variety of histological techniques to characterize the adult structure of the olfactory system in the adult zebrafish. They concluded that the structures and the synapses observed in the olfactory bulb of this fish are typical of what is found in other vertebrates.

Fishelson (1995) compared the morphology and cytology of the olfactory organs in moray eels (*Muraenidae*). They found that, as in other teleosts, the lamellae in them are covered by a ciliated

epithelium composed of three types of sensory cells : Ciliated sensory neurons, ciliated supporting cells and sensory cells which bears microvillae as well as cilia. The proximal axonal extensions of the ciliated cells cross the basal lamina in bundles and combine to form fila olfactoria from which the two olfactory nerves extends towards the olfactory bulbs. Lateral extensions at the basal parts of these ciliated cells, the so called spines, cross the membranes of neighbouring cells as dendrites, possibly changing part or all of the ciliated epithelium into an olfactory field. The density and number of sensory cells on the lamellae, as well as observed differences in their foraging behaviour in nature and captivity, enable the morays to be divided into two groups : one group, in which the lamellae are richly covered with stereocilia, includes species, that search for food by olfaction ; and the second group, which has a great deal less cells with stereocilia and includes the species, that locates its food visually.

Singh *et al.* (1996) described the olfactory organ of *Ilisha motius* (Ham). They reported oval olfactory rosette bearing club shaped median raphe and quadrangular shape of lamellae.

Fishelson and Baranes (1997) studied the ontogenesis and cytomorphology of the nasal olfactory organs in the Oman shark, *Iago omanesis* (Triakidae). Olfaction in them is one of the central senses by which they forage, especially at night and in deep water. The organs responsible for this function are the olfactory rosettes, which are situated in their nares. In new born and adult fish the nasal olfactory organs are composed of olfactory lamellae with secondary folds. Ontogenesis of the nasal rosettes is characterized by a gradual

development of the lamellae and their secondary folds, with a concomitant ripening of the sensory elements (Ciliated, microvillar and rod like bearing cells), as well as glandular and supporting cells and cells containing kinocilia that agitate the nasal water flow. Ciliated and rod bearing sensory neurons are described for the first time in sharks.

Zielinski and Hara (1998) investigated the morphological and physiological development of olfactory receptor cells in rainbow trout embryos. They concluded that in rainbow trout the olfactory receptor cell has two separate morphological forms, ciliated and microvillar. These are ontogenetically distinct and the ciliated receptor cells preceded the microvillar. They also demonstrated that the ciliated receptor cells respond to amino acid stimulation.

Calzada *et al.* (1998) studied the developmental stages of the larvae of *Spanis aurala* and reported ciliated and non-ciliated cells along with mucous secretory activity in submucosal zone.

Liang *et al.* (1998) conducted their experiment to identify the role of sense organs in the feeding behaviour of chinese perch, *Siniperca chuatsi*, by determining the consumption of natural food after selective removal or blocking of eyes, lateral line and olfactory organs, and also by observing the behavioural response to visual, mechanical and chemical stimulation by artificial prey.

Sharma *et al.* (1999) reported the abundance of aquatic resources, diversity of species, compactability with their forming system and an upcoming activity. Histoecological study of olfactory organ will also demonstrate to sketch out such sustainability in bio-

physico-chemical variables encountering in the habitat of a particular fish.

Gautam and Gautam (2000, 2001, and 2002) specifically demonstrated the effects of pesticide and different toxic contents present in water on the cellular composition of gastrointestinal.

Such effects may be visualized in the olfactory mucosa too, as water is constantly circulating through the olfactory chamber, which may be bearing some pollutants. Now a days no water is free from pollutants and histoeologically the olfactory epithelium is so designed to get it automatically protected by such pollutants effects. The present study will emphatically demonstrates the role of histological components of olfactory epithelium and its different formations which neutralizes the pollutant effects and also create enhancement in the receptory surface of olfactory mucosa.

Liao and Chang (2003) studied the role of sensory mechanisms in predatory feeding behaviour of juvenile red drum, *Sciaenops ocellatus*. They focused on predation responses influenced by vision, olfaction and lateral line in captive juvenile red drum. They concluded that mechanoreception plays the primary role and vision the secondary role in predatory behaviour of the red drum.

Belanger *et al.* (2003) comprehensively studied the morphology and histochemistry of the peripheral organ in the round goby, *Neogobius melanostomus*. In them, the location of olfactory mucosa within the olfactory chamber is novel for teleost fish, as it extends beyond the ventral surface to the lateral and dorsal regions. The widespread occurrence of olfactory sensory neurons in the olfactory

chamber supports the idea that olfactory signaling is important to the survival of the round goby. The prominence of the lachrymal and ethmoidal accessory nasal sacs indicates the capacity to regulate the flow of odorant molecules over the sensory surface of the olfactory sensory neurons, possibly through a pump like mechanism driven by opercular activity associated with gill ventilation.

Eastman and Lannoo (2001, 2003 and 2004) and Lannoo and Eastman (2005) examined the morphology, anatomy and histology of brain and sense organs of Antarctic eel cod (*Muraenolepis microps*), Antarctic plunderfish (*Dolloidraco longedorsalis*), Antarctic ice fishes and Antarctic eelpouts, to describe the sense organs and its relation with the brain from all perceptory aspects. They demonstrated that all above fishes possesses olfactory rosette with lamellae and nasal sac supports water supply to the olfactory system. They also described that the proximal axonal extensions of the ciliated cells cross the basal lamina in bundles and combine to form fila olfactoria from which two olfactory nerves extends towards the olfactory bulbs.

The length of the olfactory nerve varies greatly depending upon the relative position of the olfactory bulb in different types. Three types of positions have been recognised :

- (i) If the bulbs are located close to noses, the olfactory nerves are very short and a long olfactory tract is present. Such condition is referred as pedunculated. This type of condition is found in all elasmobranchs, in *carassius*, *Ictalurus* and other cyprinids, cobitids, silurids etc.

- (ii) If the bulbs are close to hemispheres of the forebrain, than olfactory nerves are found to be long. This condition is referred as sessile. Examples are *Anguilla*, *Esox*, *Salmo* and the majority of telosts;
- (iii) The third condition exists, when the position of the bulbs is intermediate between nose and forebrain. This has been found in *Raniceps raninus* and in *Gymnothorax kidako* and *Coryphaena hippurus*.

Singh and Sinha (2006) described the morphology and anatomy of the olfactory organ of a hill stream fish, *Sisor rhabdophorous* (Ham.) The olfactory rosette in them is elongated in shape and can be placed under Burne's rosette column II or with Bateson's rosette type 2. They also reported that, the number of lamellae in the rosette increases with the growth of the fish and new lamellae are always added at its anterior end.

Histochemistry is a recent creation, expressing a profound aspiration, the recognition in the cell, by means of appropriate chemical reaction of different product formed during the life. Vass (1952) had invented the term histopochemistry specifically to signify the prime concern of histochemistry with localization. The term histochemistry now contains cytochemistry as its premier division, despite the view of Gomori (1952) that the name of later portion should be reserved for studies on the chemical organisation of cells in general.

We have already seen that amino acids and related compounds which are normally nonodorous to humans, are one of the major

active components to elicit behavioural changes through olfaction in many fish species (Olmsted, 1918 Idler *et al.* 1956, 1961; Steven, 1959; Kleerekoper and Mongensen, 1959, 1963; Hoese and Hoese 1967; Hashimoto *et al.* 1968; Konosu *et al.* 1968; Tucker and Suzuki, 1972; Pfeiffer and Lemke, 1973). Furthermore, recent electrophysiological studies show that certain amino acids are extremely effective olfactory stimuli and may play an important role (Sutterlin and sutterlin, 1971; Hara, 1972, 1973, 2006;) Saglio *et al.* 1990).

Adrian (1950) suggested that olfactory discrimination depends mainly on spatial organization of the receptors. Kistiakowsky (1950) postulated that the phenomenon of perception of odour would be attributed to differential inhibition of the component enzyme or enzymes in these systems by olfaction exciting substances. Baradi and Bourne (1951) demonstrated the role of enzymes in olfaction in the higher vertebrates (Mammals) and suggested that odorous substances might act by inhibiting the enzymes in a differential fashion. Davies (1962) proposed yet another hypothesis and according to him the mechanism of olfaction lies in the penetration and dislocation of a small region of the wall of an olfactory nerve cell. This dislocation allows the K^+ and Na^+ ions to move across the membrane, initiating the nervous impulse. Prosser (1962) postulated that a unified theory of chemical stimulation is very difficult because chemoreception is partly related to the permeability of cell surfaces, partly to adsorption and partly to chemical reactions at surfaces. Doving (1966) reported that the chemicals least effective in evoking excitatory or inhibitory responses were p-chlorophenol, β -ionone, and

menthol and the most effective chemicals were fumaric acid, eugenol, pentamethylenediamine, glutathione and coumarin. Non-volatile substances does not evoke sensation of smell. However, nonvolatile substances may evoke a sensation of smell, when introduced into the olfactory epithelium in solution (Backman 1917 ; Doving, 1966).

Munshi and Singh (1975) studied the histochemical observations on the olfactory glands and the olfactory epithelium in *Channa punctata*. He applied histophysiological approach to analyse the mechanism of olfaction in this fish. Stimulating chemicals such as glutathione, fumaric acid, coumarin, β -ionone and P-chlorophenol were used at a concentration of 10^{-3} M in distilled water. Tap water and odour free distilled water flushed olfactory organs were used as controls. While strong alkaline phosphatase reactions were obtained in the sensory hairs and on the surface of the olfactory epithelium of the control, there is more or less complete inhibition of the same in the experimental ones except coumarin treated fishes. There were some reactions for alkaline phosphatase in β -ionone and glutathione treated olfactory epithelium.

From the limited literature available on histochemistry, Bronshtein (1965) concluded, that, the mosaic nature of the spatial distribution of chemical and biologically active substances is very distinct in the body and processes of the olfactory cells of higher vertebrates, being less pronounced in those of cyclostomes and telostei, in which they are more evenly distributed over the body and on entire peripheral processes of the olfactory cells.

Cinar and Senol (1989) during histochemical analysis of the intestine of flower fish, *Pseudophoxinus antalyae*, showed that the gastrointestinal mucous content include sulphate-esters and/or carboxylic, glycogen and / or oxidable dioles, neutral or acid rich sialic acid residues and strong acid sulphated glycoproteins.

Similar findings were also reported by Marchetti *et al.* (2006) during histochemical analysis of the entire alimentary canal of the rainbow trout, *Oncorhynchus mykiss*.

Kozaric *et al.* (2006) reported histochemical distribution of digestive enzymes in intestine of goldline, *Sarpa salpa*. They investigated histochemical localization of non specific esterase, alkaline and acid phosphatase in the intestine of free living gold line.

Chapter-2

Material and Methods

Material and Methods

Large number of living *Cyprinus carpio* of different sizes were obtained from Konch, Jalaun district, U.P. *Tilapia mossambica* were procured in living condition from Arjun Tal in Charkhari, Mahoba district, U.P. *Bagarius bagarius* were procured in living condition from Betwa basin, Pahooj reservoir, Jhansi district, U.P.

The fishes collected in living condition were kept in aquarium for experimental use.

For histological studies, adults of proposed fishes were anaesthetized in fresh solution of MS 22 (1gm MS 222, 3000 ml water) and their olfactory rosettes were dissected out in the Ringers solution and fixed in Bouin's fluid and Lillie's neutral buffered formaline. They were processed by routine method and embedded in paraffin at 56-58°C. Serial transverse and horizontal sections were cut of 6-8 μ m thickness and stained in Iron allum haematoxyline and counter stained by Eosin.

Specific demonstration of shape and types of olfactory epithelial cells was done through vital staining by Trypan blue method employed by Holl (1965), Chempol's (Czech) Trypan Blue. The olfactory rosettes from anaesthetized fish were treated separately for (a) 20 min in 0.25% solution of trypan blue in distilled water, (b) 40 ml in 0.5% concentration of trypan blue in distilled water, (c) 60 min. in 1% concentration of trypan blue in (i) distilled water (ii) 0.7% sodium chloride solution. The stained tissues were fixed in Heidenhein's susa iso-propyl alcohol.

For histological sections, the tissue was embedded in paraffin at 56-58°C after processing through Methyl Benzoate and Benzene. Serial transverse and horizontal sections were cut of 6-8 μ m thickness.

For the localization of histochemical content in the cellular components, special fixatives and stains were used.

[A] Method for phosphatase :

- (I) Calcium- Cobalt method for alkaline phosphatase (Gomori, 1952; Lillie, 1954).

The alkaline phosphatase hydrolysis a variety of monoorthophosphatase esters in an alkaline medium (pH 9.0). The rate of hydrolysis is increased by the presence of magnesium. The alkaline phosphatase is also called as "Phosphomonoesterase I". At PH 9.0 phosphate ions are liberated from the substrate glycerophosphate by the action of the enzyme and are immediately precipitated as calcium phosphate. Then the calcium is substituted for cobalt and shown as opaque cobalt sulphide. In this method, a false positive reaction is given by calcium, that is already present in the tissue, and by other black pigment such as carbon. Controlled sections subjected to distruction of the enzyme activity by heat (floride were not used) or using distilled water in place of substrate, were taken through the technique with the test sections. In test sections, the blackenings at sites that were not in the control sections are taken to represent the alkaline phosphatase activity.

For fixation, cold Acetone (4°C) was used for paraffin section. A rosette of olfactory organ was fixed in acetone for 24 hours at 4°C in

refrigerator. The dehydration of the material was done with acetone, cleared with two changes of benzene for 30-60 minutes each and impregnated with paraffin wax for 30 minutes in a vacuum over 56°C. Sections were cut at 6 μ m thickness, flattened on lukewarm water and mounted on albumenized slides. The staining of sections was done in following steps .

- (a) Brought sections in distilled water.
- (b) Incubate the section in working substrate solution for 1 hr.
- (c) Washed in distilled water for 4 minutes.
- (d) Treated with 2% aqueous cobalt nitrate for 5 minutes.
- (e) Washed in several changes of distilled water for 6-8 minutes.
- (f) Treated with fresh 1% solution of yellow ammonium sulphide for about 1 minute.
- (g) Washed in running tap water for 5 minutes.
- (h) Counter stained for 2 minutes with 0.1% safranin in 0.1% acetic acid.
- (i) Quickly dehydrated with alcohol, cleared in xylene and mounted in synthetic resin medium.

The sites of alkaline phosphatase activity were found brown to black. Calcium present in the tissue was also black.

(II) : Lead Nitrate Method for Acid phosphatase :

The acid phosphatase or "phosphomonoesterase-II" splits into mono-orthophosphatase esters in acidic medium (optimum PH is usually around 5.0). It is inhibited by fluoride and not activated by magnesium.

In the lead nitrate method, the enzyme acts upon the substrate of organic phosphate in an incubating medium containing a salt of lead. The phosphate forms a lead phosphate. The lead phosphate is subsequently converted into opaque lead sulphide by ammonium sulphide.

The fixation of the olfactory epithelia was done in cold acetone for paraffin sections.

The technique of the staining is followed as given below :-

- (a) Brought the sections in distilled water.
- (b) Incubate the substrate solution for 4 hrs. at 37°C.
- (c) Washed briefly in distilled water and kept in a dilute (1% or less) fresh solution of yellow ammonium sulphide for 2 minutes.
- (d) Washed the section in tap water and counter stained with 1% aqueous eosin for 5 minutes.
- (e) Washed the section in tap water and distilled water and mount it in a glycerine jelly.

[B] Method for Glycogen :

It is no longer necessary to fix glycogen in absolute alcohol or picric acid, but the glycogen is fixed immediately. Fixation of glycogen was done with picric acid in 96% alcohol (85 parts 40% formaline (10 parts) and glacial acetic acid (5 parts). Periodic acid schiff (PAS) reaction was used in the laboratory for glycogen demonstration. According to this reaction, the adjacent 1:2, 1:2 glycol groups (CHOH-CHOH) are broken by periodic acid into aldehydes (two CHO group), these are demonstrated with schiff's reagent.

The Procedure used for staining as follows :-

- (a) One section was treated as below :
 - (i) Taken to water.
 - (ii) Treated with 0.1% freshly prepared, malt diastase solution in distilled water at 37°C for 30 minutes.
 - (iii) Thoroughly washed in running water for 5 minutes.
- (b) The other section was left in distilled water for a similar duration in place of step (ii) given above.
- (c) Stain both the sections by picric acid schiff reaction.

In result, the PAS positive material that was seen in untreated section absent in the digested section may be assumed to be glycogen.

[C] Method for Lipid :

The Sudan Black B method was followed for localization of lipid in the olfactory epithelia. For this experiment, saturated 100 ml of acetone with sudan black B and an equal volume of 70% alcohol is taken. Allowed to stand, filtered and stored in tightly stoppered bottle for staining.

The steps used for staining are given below :-

- (a) Brought sections in 70% alcohol.
- (b) Immersed in stain (5-10 min).
- (c) Transfer to 70% alcohol.
- (d) Submerged in tap water.
- (e) Washed in distilled water.
- (f) Mounted in aqueous medium.

The black pigment were observed in the result.

[D] Method for Acid Mucopolysaccharides :

For localization of acid mucopolysaccharides, Alcian Blue method was employed. The Alcian blue is a copper phthalocyanin dye

compound and at low pH stains acid (including sulphate) mucopolysaccharides by salt linkage with acid group.

The steps used for staining are given below :-

- (a) Take paraffin sections and dewax in Xylene for 10-15 minutes.
- (b) Brought the section to water through descending grades of alcohol.
- (c) Stained in Alcian Blue solution for 20 minutes.
- (d) Rinsed in distilled water and then washed with tap water.
- (e) Dehydrated through ascending grades of alcohol.
- (f) Cleared in Xylene and mounted in D.P.X.

Blue stain of acid Mucopolysaccharides were found in result.

[E] Method for metacromasia :

For localization of metacromasia the materials were fixed in 10% formaline and Toluidine Blue method was employed for localization.

The method of staining is given below :-

- (a) Brought sections in water.
- (b) Kept in Toluidine blue solution for second.
- (c) Rinsed the slide in distilled water.
- (d) Mounted in glycerine.

Result :

- (i) In result the metachromatic substance are found to be Red, Pink or purple.
- (ii) Nuclei and other compound are found to be blue.

Chapter-3
Observations

opening

opening

lateral side

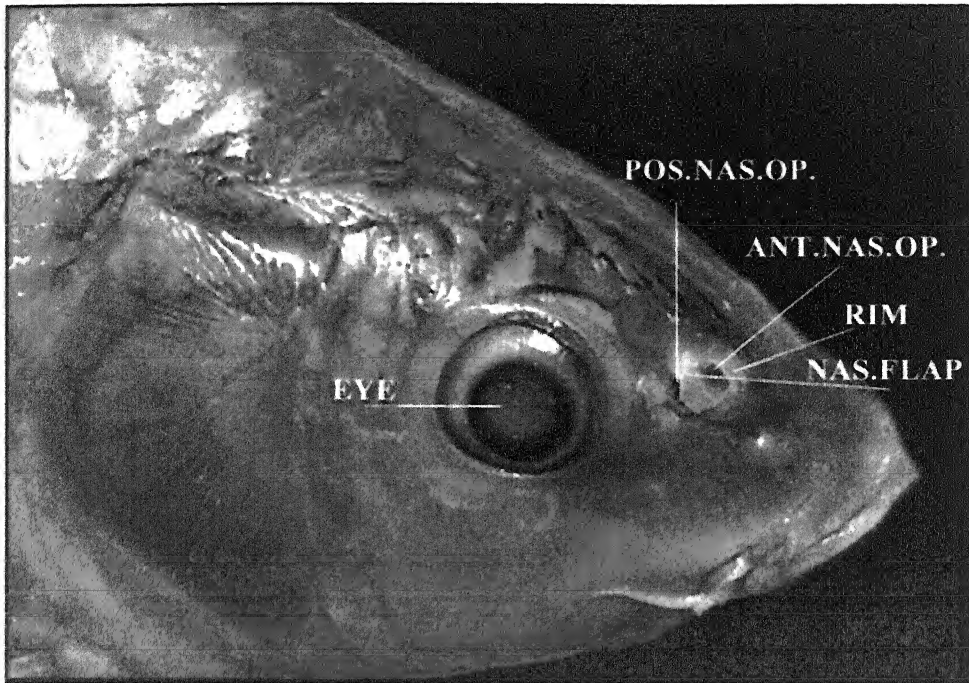


Plate.1

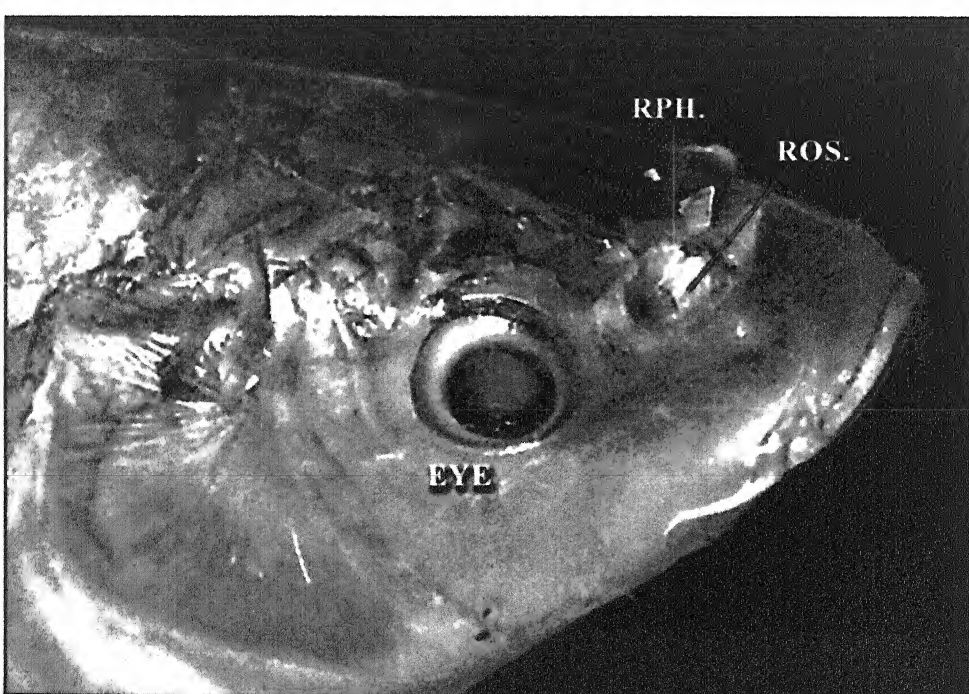


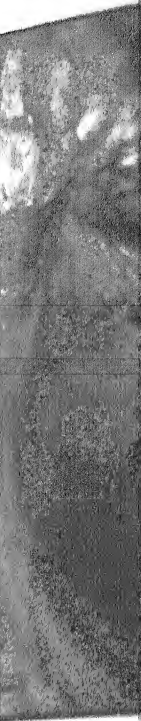
Plate.2

Plate-1 : Lateral view of head of *C. carpio*

ANT. NAS. OP.	-	Anterior nasal opening
Eye	-	Eye
NAS. FLAP	-	Nasal Flap
POST. NAS.OP.	-	Posterior nasal opening
RIM	-	Rim

Plate-2 : Dissection of the head of *C. carpio* from lateral side to show rosette insitu

ROS.	-	Rosette
RPH.	-	Raphe



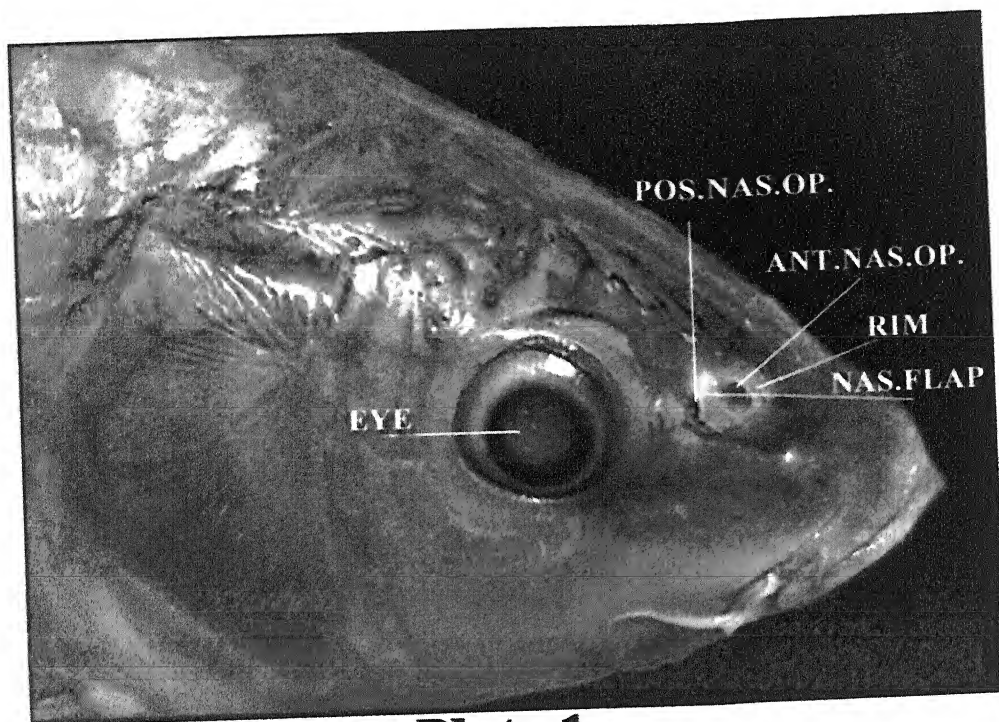


Plate.1

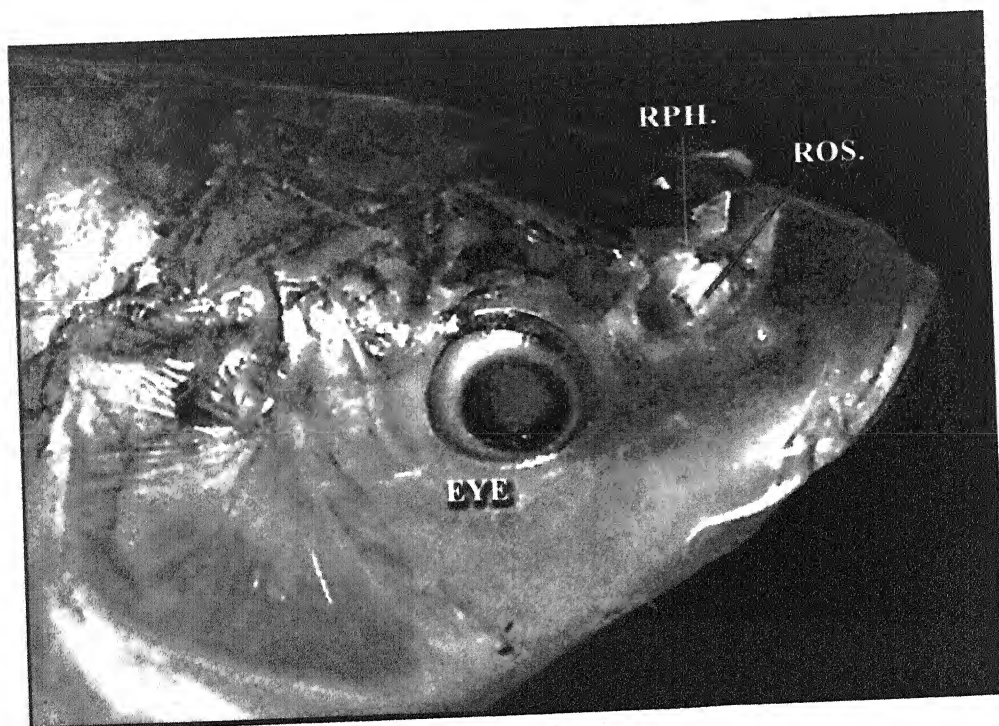


Plate.2

Histological Observations of the Olfactory Organ of *Cyprinus carpio* Linnaeus

C. carpio bears a pair of olfactory chambers (OLF. CHAM.) lying on the dorso-lateral surface of the head and are more close to the eye-orbit than the snout (Plate-1, Fig.-1A). The olfactory chambers are oval in shape and get surrounded by integumental formation, which forms an upwardly and forwardly erected nasal flap (NAS. FLAP, Plate-1, 2); Figs.-1A, 1B). It is dipped into the olfactory cavity by its ventral extension, dividing it transversely in the anterior and posterior chambers (Figs. 1A, 1B, 1C). The olfactory chamber is communicated outside by a pair of nasal openings which lie close to each other. The nasal flap act as partition in between them (apertures). The nasal openings allow most of the part of the olfactory chamber exposed to water except that covered by the integumental borders of nasal flap. The rosette can be seen easily through the posterior nasal opening (POS. NAS. OP., Figs. 1A, 1B, 1C).

The olfactory rosette (ROS.) is oval shaped and occupies the entire olfactory chamber (Fig. 1D). It has a ventral convex and dorsal concave surface with large number of closely set lamellae (LAM., Fig. 1D). A leaf shaped thick raphe (RPH.) divides the olfactory rosette in ethmoidal and lacrymal halves and extends antero-posteriorly of the rosette. (Plate-2, Fig. 1D) In the extreme periphery of the lacrymal half, the olfactory epithelium remains lamellaeless (LAM. LESS. AREA) forming a pocket like structure which probably be understood as rudimentary accessory sac (Fig. 1D). This may help in retaining water during the course of its transportation from the olfactory chamber.

- Fig.-1A : Diagram of the lateral view of the head of *C. carpio* .
- Fig.1B : Diagram of the olfactory chamber to show nasal flap and posterior nasal opening in *C. carpio*.
- Fig. 1C : Diagram after removing the nasal flap to show the position of anterior nasal opening and RIM in *C. carpio*.
- Fig. 1D : Diagrammatic sketch of the rosette of *C. carpio*.
- Fig. 1 E: A set of 1 - 18 lamellae from one half of the rosette of *C. carpio*.

ANT	:	Anterior
ANT. NAS. OP	:	Anterior nasal opening'
CEH. CH	:	Central Channel
ETH. H.	:	Ethmoidal half
EY.	:	Eye
INT. LAM. SP	:	Inter lamellar space
LAC. H.	:	Lacrymal half.
LAM.	:	Lamella
LAM. LESS AREA :		Lamellaeless - Area
LING. P.	:	Linguiform Process
NAS. FLAP	:	Nasal flap
OLF. CHAM.	:	Olfactory chamber
PER. CH.	:	Peripheral Channel

POS. NAS. OP.
VEN. EX. FLAP
NAS. FLAP
OLF. CHAM.



5mm

1

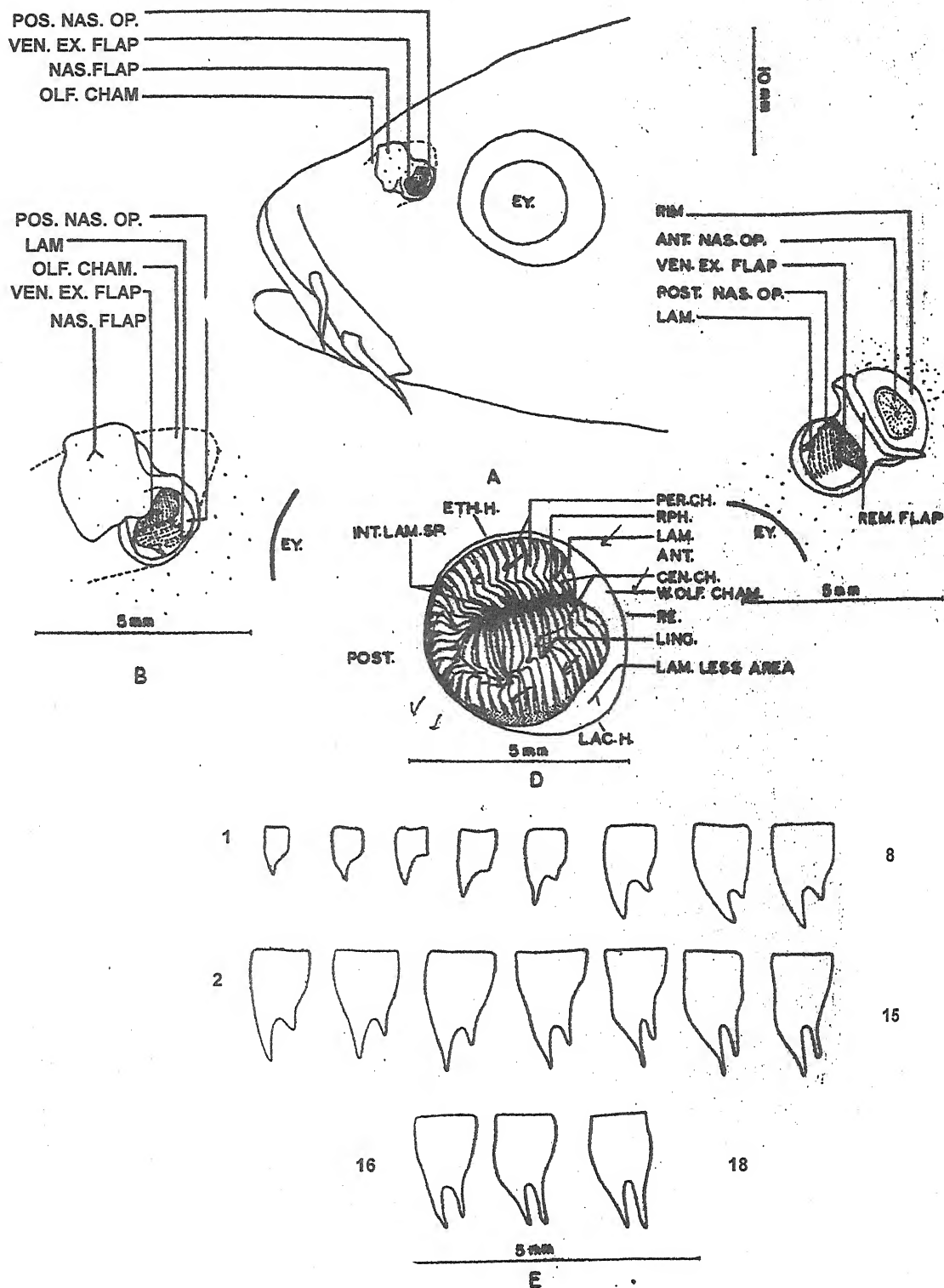


Fig. - 1

Fig.-2 : Diagram of the dissection of the head of *C. carpio* from dorsal side to show the relationship of brain with rosette .

CE.	:	Cerebellum
EY.	:	Eye
OLF. BL	:	Olfactory bulb
OLF. LO	:	Olfactory load
OLF. TR.	:	Olfactory tract
OP. LO	:	Optic Load
ROS.	:	Rosette

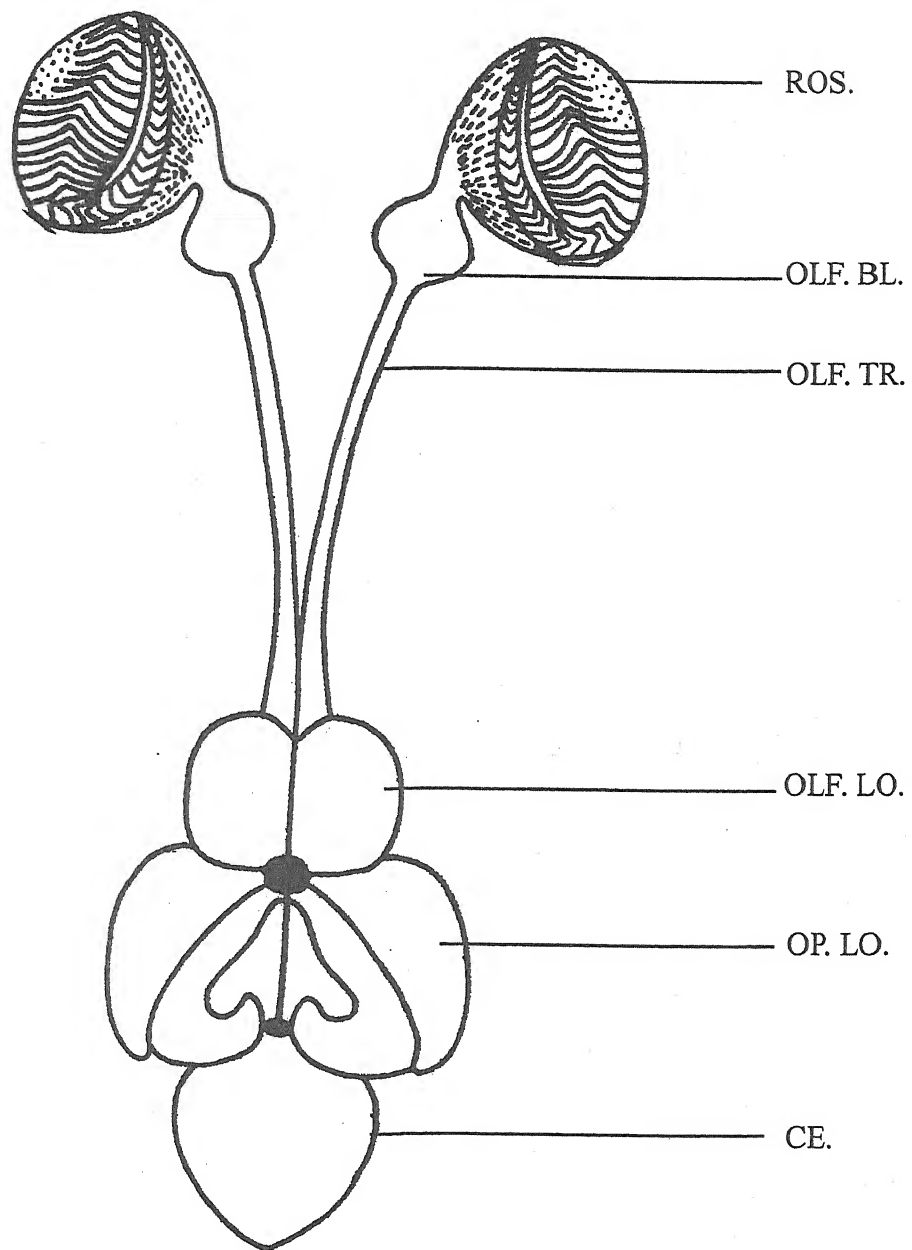


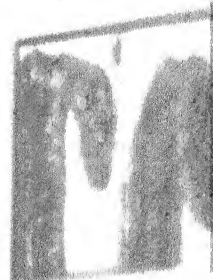
Fig. - 2

Each half of the rosette is further divided into peripheral and central channels due to presence of linguiform process of all the lamellae in an antero-posteriorly progressing manner. The linguiform processes (LING.P.) form a curtain like separation in between the channels of each half of the rosette (Fig.-1D). The raphe is richly supplied with chromatophores but in other regions of rosette they are scattered rarely.

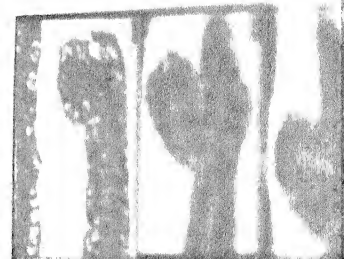
The lamellae (LAM., Fig.-1E) are leaf shaped structures lying attached on either sides of the raphe (Fig.-1D, Plates-3, 4). They are possessing ventral convex and dorsal flat surface. The former is attached with the wall of olfactory chamber where as latter is free and maintain *interlamallar* spaces (INT.LAM.SP., W.OLF.CHAM.,Plate-5) among them. The proximal end (PRO.E.) of each lamellae is narrow and attached with the raphe while the distal end (DIS.E.) is broad and attached with olfactory chamber (Plates-3, 4, 5). The linguiform process is present in the middle of each lamellae and are arranged in an antero-posterior ascending series. In few posterior lamellae its growth exceeds beyond the distal end of the lamellae (Fig.-1E, Plates-6-18). The chromatophores are present on the linguiform process (Fig.-1E).

The brain and its cranial connections are exposed after dissecting the fish from dorsal side and removing the frontal and partials. The olfactory bulbs (OLF.B.) are conspicuous and bulbous structures, against the convex surface of the olfactory rosette. It receives the olfactory nerve fibres from the rosette and joins the hemisphere of forebrain by a thick olfactory tract (OLF.TR.). The

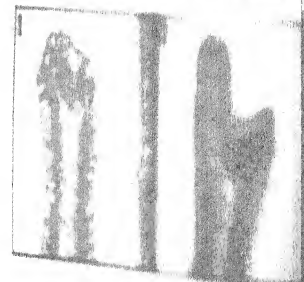
Plate-19: The distal end of lamella showing terminal bud formation, trifurcation and bifurcation.



A. Terminal bud formation



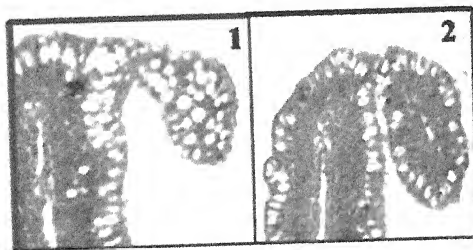
B. Trifurcation series



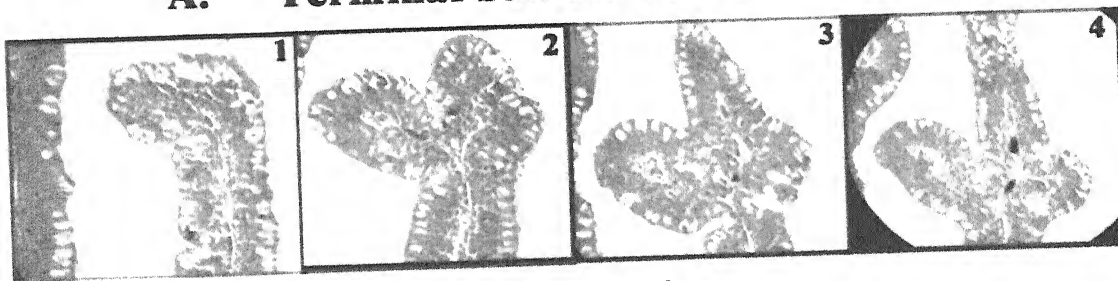
C. Bifurcation series

100-10

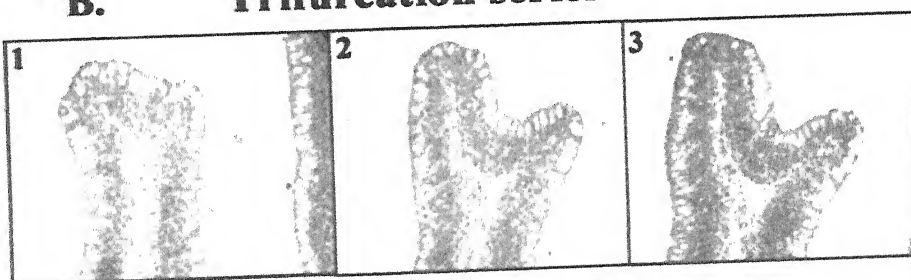
WATER TERMINAL



A. Terminal bud formation



B. Trifurcation series



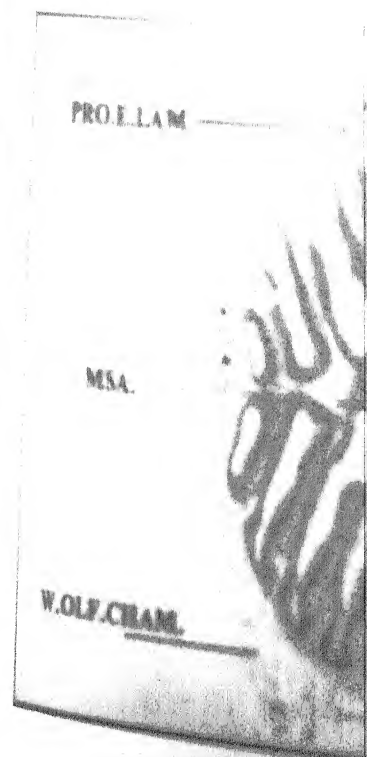
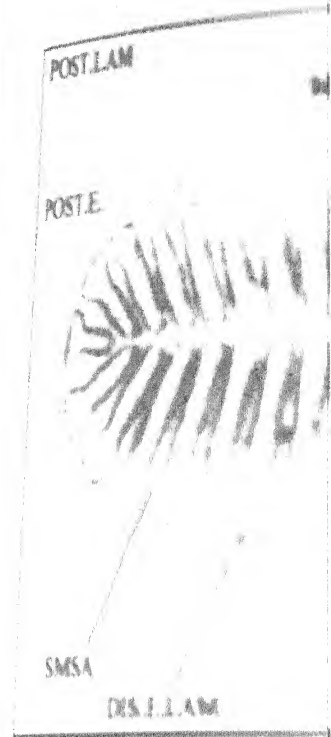
C. Bifurcation series

Plate-3 : Horizontal section of complete rosette of *C. carpio* showing the lamellar arrangement in relation to raphe and with olfactory chamber. The distal end of lamella makes a continuous series forming a peripheral channel for water circulation. Central channel is on both sides of the raphe for water circulation Magnification 50X.

ANT. E.	-	Anterior end
DIS.E. LAM.	-	Distal end of lamella
INT.LAM.SP.	-	Inter lamellar space
MID.LAM.	-	Middle lamella
POST.E.	-	Posterior end
POST.LAM.	-	posterior lamella
PRO.E.LAM.	-	Proximal end of lamella
RPH.	-	Raphe
SMSA.	-	Sub mucosa

Plate-4 : Transverse section T.S. of lamellae of *C. carpio* showing emergence of raphe and lamellar attachment with olfactory wall.

BIF.	-	Bifurcation
DIS.E. LAM.	-	Distal end of lamella
MSA.	-	Mucosa
PRO.E.LAM.	-	Proximal end of lamella
RPH.	-	Raphe
SMSA.	-	Submucosa
W.OLF.CHAM.	-	Wall of olfactory chamber



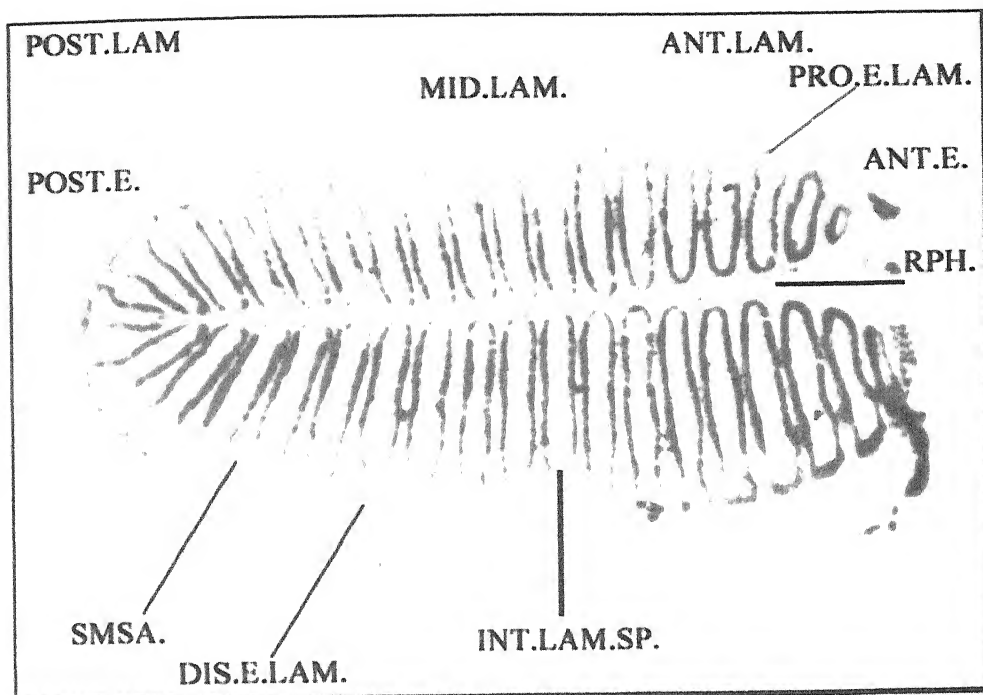


Plate.3

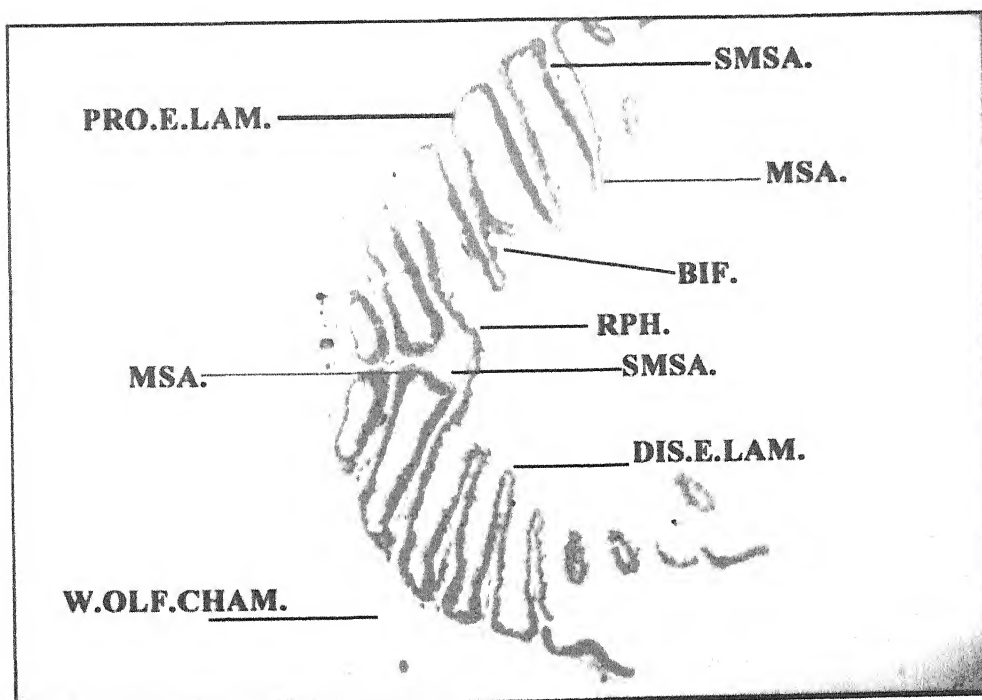


Plate.4

Plate-5 : Horizontal section of rosette of *C. carpio* showing one half of lamellar arrangement with raphe and with olfactory chamber. Peripheral goblet cell are seen occupying whole of the lamellar surface except few intervening supporting cells and receptors. Connective tissue, blood and nervous supply is through submucosa of raphe to the submucosa of lamella. Magnification 100 X.

ARE.	-	Areolae
BM.	-	Basal membrane
DIS.E. LAM.	-	Distal end of lamella
GC.	-	Goblet cell
INT.LAM.SP.	-	Inter lamellar space
MSA.	-	Mucosa
RPH.	-	Raphe
W.OLF.CHAM.	-	Wall of olfactory chamber

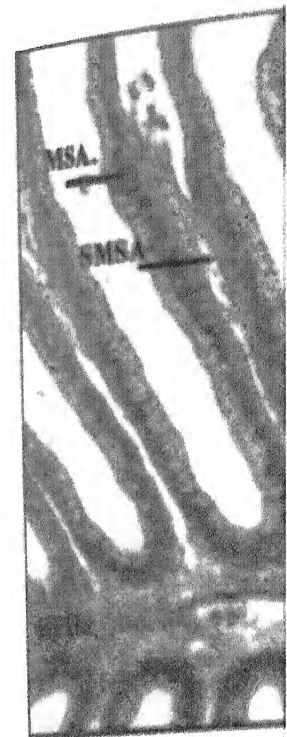
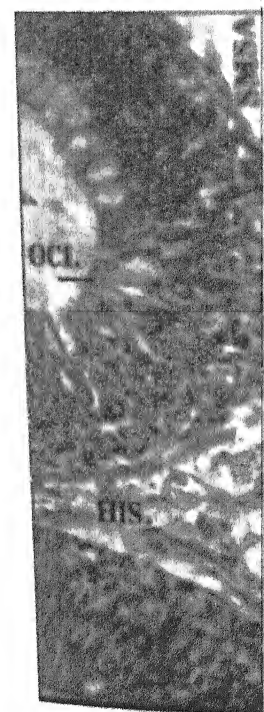


Plate-6 : Magnified horizontal section passing through the raphe of *C. carpio* showing the regular emergence of lamella along with connective tissue, fibres blood and nervous entering in submucosa of lamella. Magnification 450 X.

BCP.	-	Blood capillaries
CONN. TIS.FIB.	-	Connective tissue fibres
FIB.	-	Fibroblast
GC.	-	Goblet cell
GC.TH.	-	Goblet cell theca
HIS.	-	Histocytes
OCL.	-	Olfactory cilia
RPH.	-	Raphe



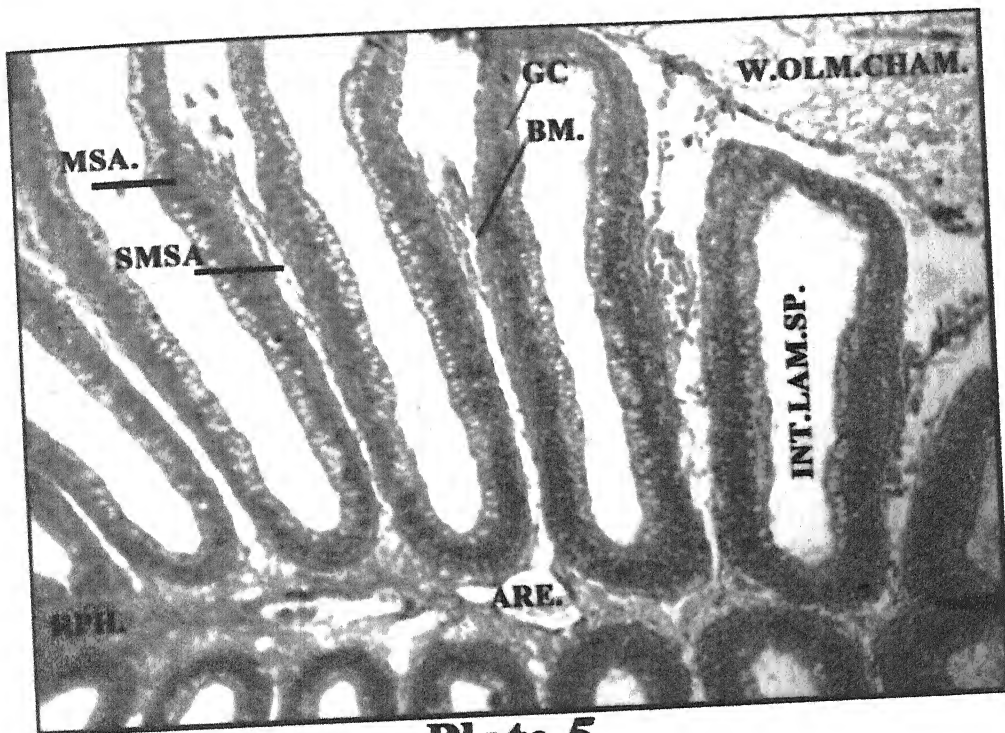


Plate.5

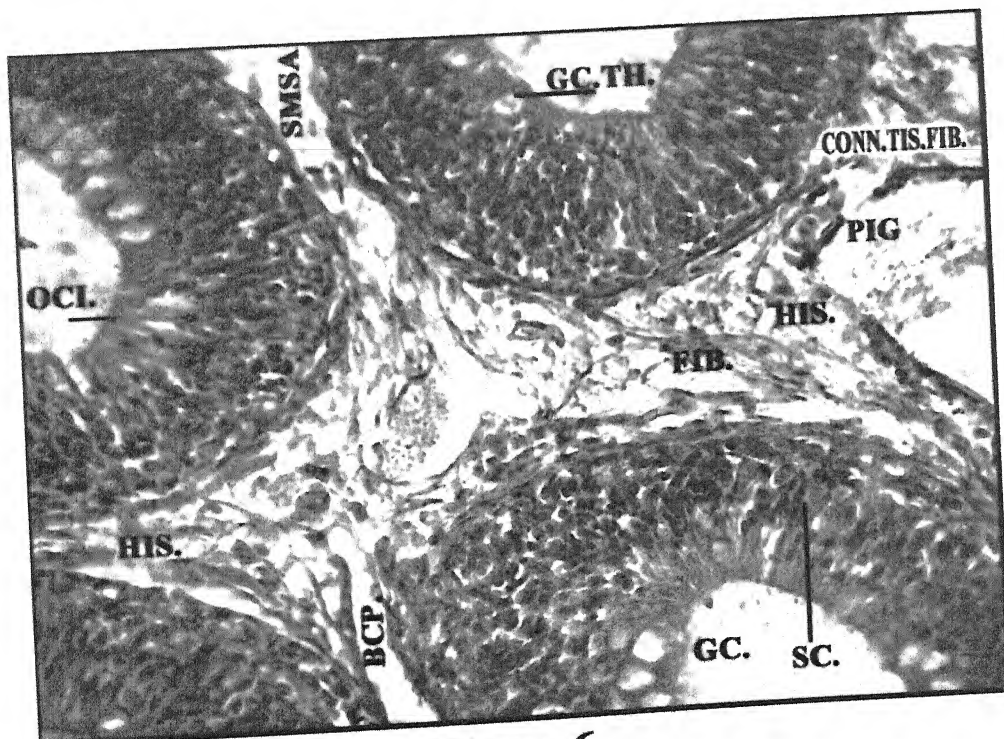


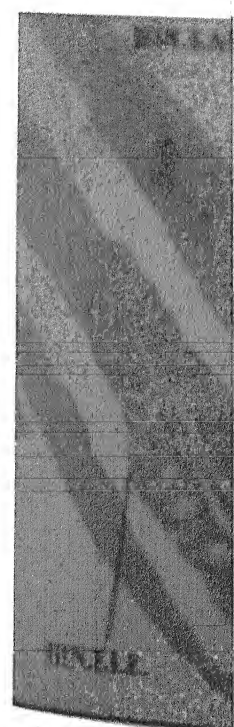
Plate.6

Plate-7 : Horizontal section through the rosette of *C. carpio* showing the sequential arrangement of initial, middle and hinder lamellae. Goblet cell activity is clearly visible in middle and hinder lamellae with the presence of crypts of different sizes at variable depths of olfactory mucosa Magnification 50 X.

DIS.E. LAM.	-	Distal end of lamella
HIN.LAM	-	Hinder lamella
INI.LAM.	-	Initial lamella
INT.LAM.SP.	-	Inter lamellar space
MID.LAM.	-	Middle lamella
PRO.E.LAM.	-	Proximal end of lamella
W.OLF.CHAM.	-	Wall of olfactory chamber

Plate-8 : Horizontal section of rosette of *C. carpio* showing middle and hinder lamellae with tremendous activity of goblet cells along with other microformations on the lamellar surface. Magnification 100 X.

CRY.	-	Crypts
HIL.ELE.	-	Hillock Elevation
HIN.LAM	-	Hinder lamella
MID.LAM.	-	Middle lamella



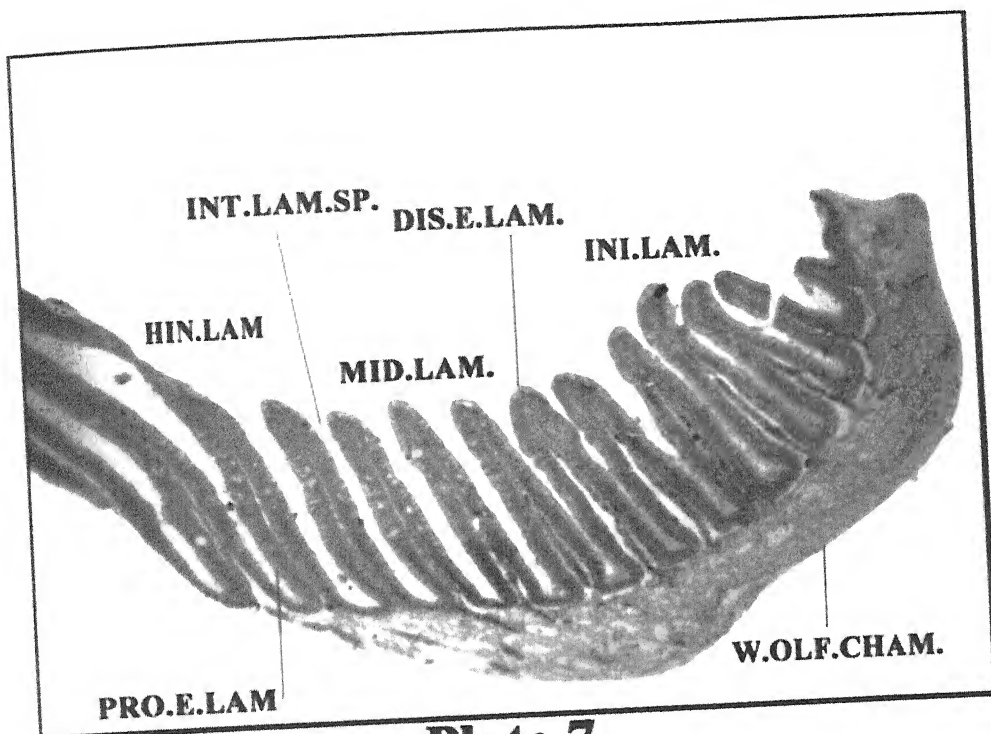


Plate.7

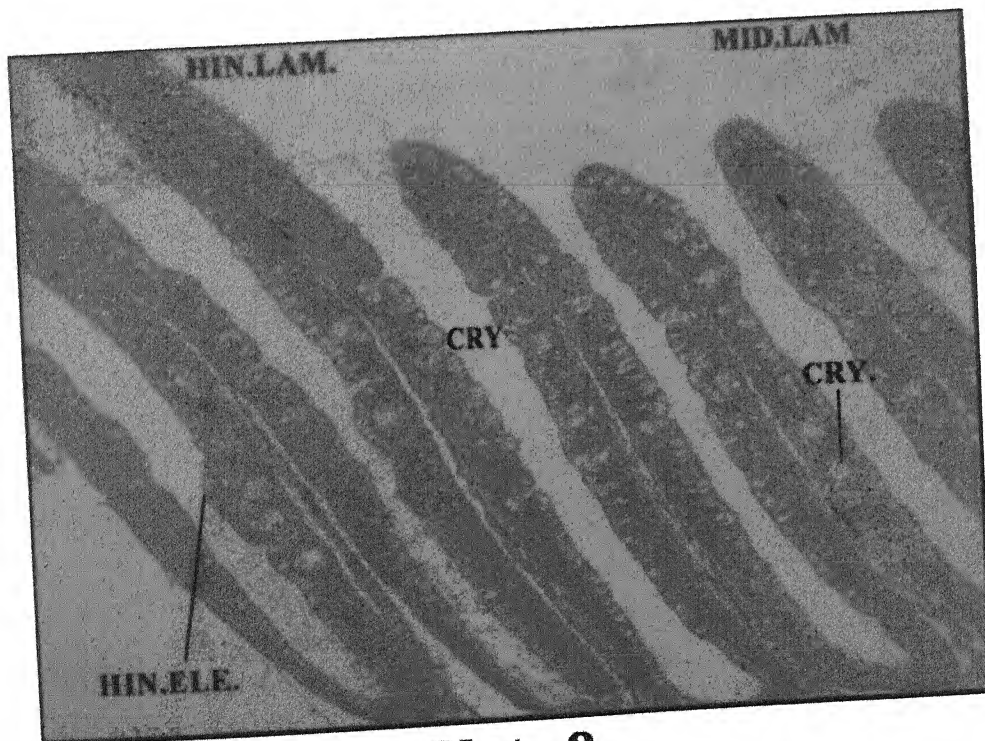


Plate.8

olfactory lobes (OLF.L.) are considerably developed but are smaller than the optic lobes (OPT.L., Fig.-2).

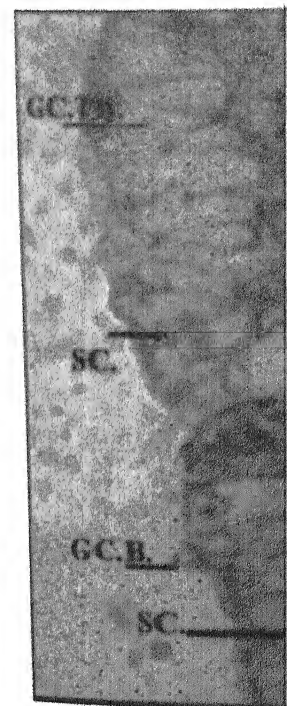
The olfactory rosette (ROS.) of *C. carpio* is oval shaped and is thrown out in number of ventro-dorsally projected folds or lamellae (Fig. 1D, Plates 3,4). They are attached on either sides of the raphe, (APH.) which is a median thickening of the olfactory floor dividing it into two equal halves (Fig-1D, Plates-2, 4, 5, 6, 10). All the lamellae are free on the dorsal surface and maintain inter lamellar spaces (INT. LAM. SP.) in between them (Fig-1D, Plates-3, 4, 5, 7, 8, 12). Each lamella is made up of a central core or submucosa, lining on its both sides by the cellular component of mucosa (MSA, Plates-3-7,9,11,12). The mucosa is composed of pseudo-stratified columnar and ciliated epithelium which is abundantly supplied with the mucous secretory goblet cells (GC., Plates-5,6,8-12). The basement membrane (BM) stands as partition in between the submucosa and mucosa (Plate-10). The peripheral surface of the lamellae is provided with number of microformations which are due to the flow of basal cells and bursting of goblet cells at different levels of the olfactory epithelium (Plates-9,10). They may be in the form of the hillock elevations (HIL. ELE.), straight projections, bifurcations (BIF.) and trifurcations (TRI., Plates-8, 17, 18). The grouping of the goblet cells and their fusion causes the interruption of the olfactory epithelium leading to the formation of depressions, flask, funnel, tubular and rounded vacuoles like crypts (CRY. Plates-14-17). The goblet cells burst on the surface in groups, forming crypts like structures on the periphery of lamellae through which receptors are projecting their olfactory cilia (OCI.) to the interlamellar space.

Plate-9 : Magnified section of terminal end of lamella of *C. carpio* showing presence of goblet cells, ciliated supporting cells, rod shaped and spindle shaped receptor cells. Magnification 450 X.

BC.Z.	-	Basal zone
DIS.E. LAM.	-	Distal end of lamella
GC.TH.	-	Goblet cell theca
MU.	-	Mucous
NU.GC.	-	Nucleus of goblet cell
RR.	-	Rod shaped receptor
SC.	-	Supporting cell
SMSA.	-	Sub mucosa
SR.	-	Spindle shaped receptor

Plate-10 : Transverse section of *C. carpio* passing through raphe zone exclusively, showing nonreceptor zone with goblet cells and nonciliated supporting cells on the peripheral margin. Submucosa of raphe is supplied with dense connective tissue fibres, blood supply histocytes, fibroblasts and mast cells. Magnification 450 X.

BCP.	-	Blood capillaries
BM.	-	Basal membrane
CONN. TIS.FIB.	-	Connective tissue fibres
FOL.OLF.	-	Folium olfactorium
GC.B.	-	Goblet cell blast
GC.TH.	-	Goblet cell theca
SC.	-	Supporting cell



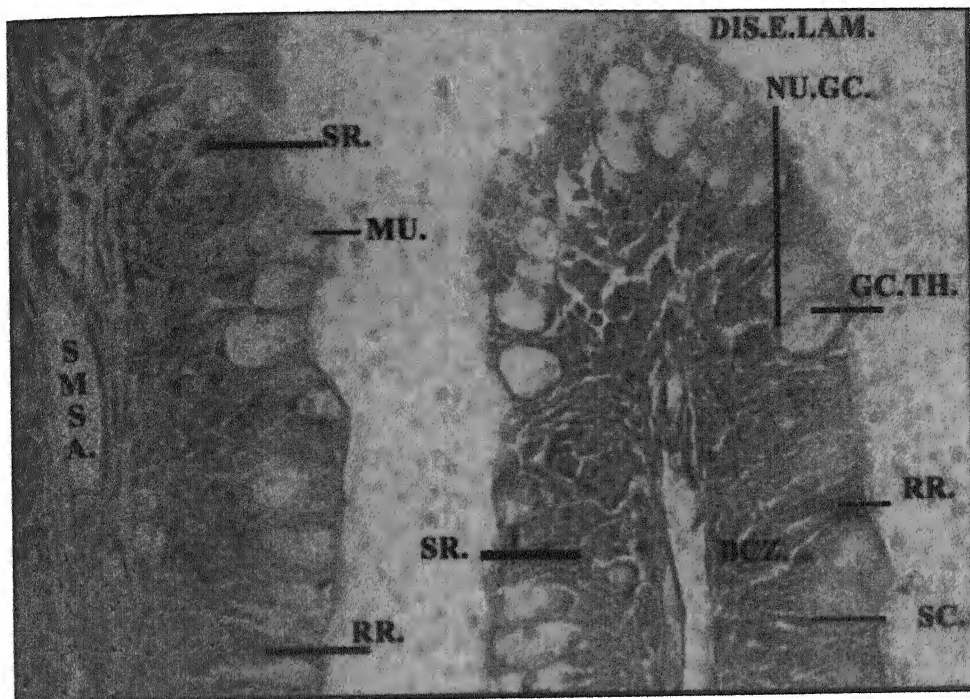


Plate.9

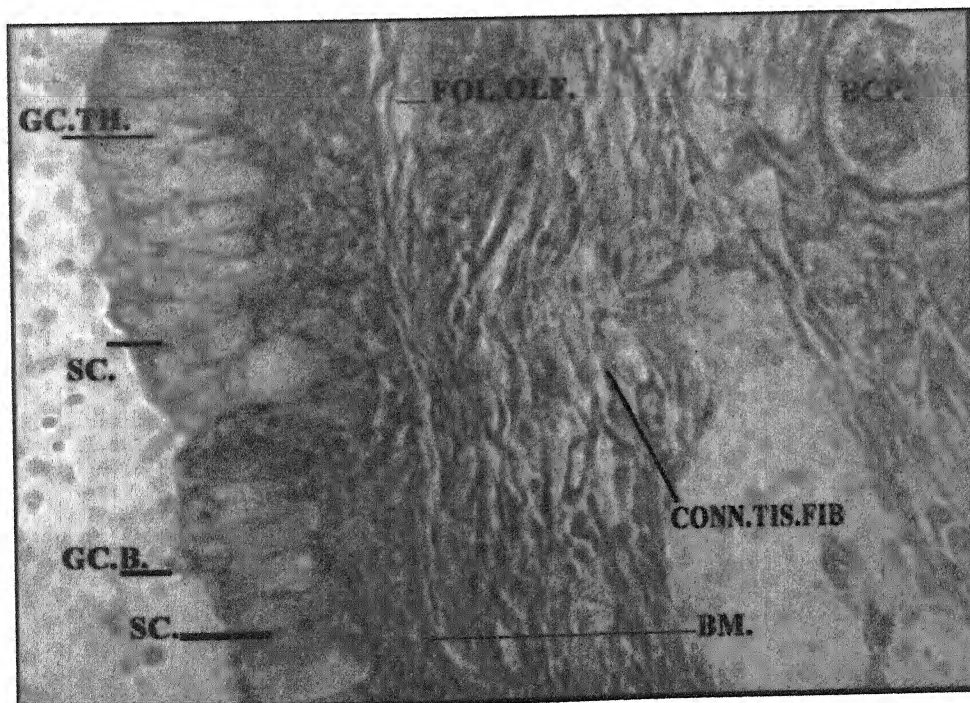


Plate.10

The crypts or opening of the goblet cells with their sensory cilia, projecting out to the inter lamellar space, gives an impression of "Olfactory Crypts", embedded deep in the olfactory epithelium (Plates-15, 16). The division of the central core or submucosa is seen only in bifurcations and trifurcations but in other micro formation it does not send its offshoots (Plates-17, 18, 20). The formation of secondary lamellae is not observed in *C. carpio* and *microformations* leads to increase the sensory surface of the olfactory lamella. Only the anterior most lamellae have their proximal and middle lamellar surface uniform (Plates-11-12) but others are richly supplied with crypts and microformations (Plates-8, 15-18). The "Cell Ball" (C. BALL, Plate-13) formation is also observed, which are arranged against the distal tip of anterior lamella.

The cellular contents of the olfactory epithelium of *C. Carpio* can be identified as : supporting or sustentacular cells, receptor cells, goblet cells and basal cells. The connective tissue of submucosa and raphae is richly supplied with branched fibroblasts, histocytes and basal cells.

Supporting cells :

The supporting cells (SC.) of *C. caripo* are subjected to a process of continuous transformation into mucous secretory goblet cells, therefore, whole of the peripheral surface of the lamella is lined by goblet cells with few intervening supporting cells (Plates-5, 9-11, 13)

The nonciliated supporting cells are present in proximal and intervening region of lamellae adjacent to raphe. These cells have elongated cell body with oval nucleus. The chromatin material is dust like and uniformly distributed in karyoplasm. The outer or distal limb

is elongated, extending upto the peripheral surface of the lamella. The ciliated supporting cells have long cilia projected into the inter lamellar spaces showing their unidirectional movement (Plate-6, 11). The distal or outer limb of the ciliated supporting cell contains homogenous cytoplasm in the distal regions of lamella. The proximal limb is inconspicuous and difficult to trace among the other cellular contents lying beneath these cells. The ciliated supporting cells are also present in crypts or opening of goblet cells among the primary neurons (Plates-9, 10).

The ciliated supporting cells in the middle and distal regions of the lamella are comparatively broad and columnar in shape with a slightly convex distal end which projects cilia in the interlamellar spaces. They bear rounded or oval nuclei with a nucleolus and faintly visible chromatin material. The nuclei of these cells are larger than the receptor cells and take darker stain as compared to primary supporting cells. The outer distal limbs of secondary ciliated supporting cells are thick and filled with fibrillar cytoplasm. The ciliation is thick and prominent, projecting into the inter lamellar space. The ciliated supporting cells may undergo a process of transformation into the goblet cell and transistional stages of these cells can easily be seen in the olfactory epithelium of *C. carpio*. Some ciliated cells are also seen discharging the mucous into the inter lamellar space at certain places.

Receptor Cells :

The receptor cells are supplied through out the olfactory epithelium of *C. carpio* irrespective of their restriction in any particular region of the lamellae. But, however, they are concentrated in crypts

and in the middle region of all the lamellae. They can be classified into three types : Primary neurons (PN.); spindle shaped receptors (SR.) and rod shaped receptors (RR.).

The primary neurons (PN.) are confined in the crypts (Plates-14-16) and in the proximal region of lamellae among the nonciliated supporting cells. They bear a rounded nucleus (NU. PN.) which send a fibrillar dendrite (DN. PN.) to the peripheral surface. The dendrite is darkly stained. These receptor cells are situated close to the basement membrane (BM.) as they usually lie in the interruptions caused by the bursting of goblet cells (GC.B.) in the form of crypts. The terminal end of primary neurons either bear cilia or protrude as such in the lumen of crypts which are communicated with interlamellar spaces by their openings. In this manner olfactory cilia (OCI., Plate-11) or protruding end of dendrite remain in contact with the water current passing through the interlamellar spaces of the lamella. The independent identity of the axon of these receptors are not very commonly traced out due to their insignificant length but, however, at the places of thick olfactory epithelium their clear demarcation can be seen.

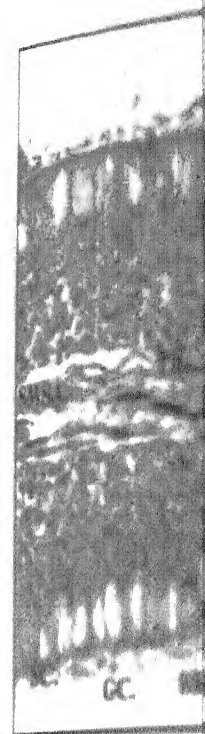
The spindle shaped receptor (SR.) bears elongated and oval nucleus (NU.SR.) with long dendrite (DN. SR.). The axonal end is also considerably long and can be easily traced out in thick regions of olfactory epithelium. Their occurrence is comparatively rare in the olfactory epithelium of *C. carpio* but, however, they can be observed among the ciliated supporting cells in thick olfactory epithelium (Plates-9, 11, 12, 14). They are not present among the marginal goblet cells or in the crypts or opening of the goblet cells.

Plate-11: Magnified section of initial lamella of *C. carpio* showing uniform mucosa and submucosa. Goblet cell occupy most of the peripheral zone along with ciliated and non-ciliated supporting cell and also with intervening rich supply of rod and spindle shaped receptors. Magnification 750X.

BCP.	-	Blood capillaries
BC.Z.	-	Basal zone
DN.R.R.	-	Dendrite of rod shaped receptor
GC.	-	Goblet cell
GC.TH.	-	Goblet cell theca
PIG.	-	Pigment cell
RR.	-	Rod shaped receptor
SC.	-	Supporting cell
SMSA.	-	Sub mucosa
SR.	-	Spindle shaped receptor

Plate-12: Magnified section of initial most lamella of *C. carpio* showing comparatively lesser goblet cell activity, intervening supporting cells, rod and spindle shaped receptors and thick basal zone. Magnification 450X.

BCP.	-	Blood capillaries
GC.TH.	-	Goblet cell theca
INT.LAM.SP.	-	Inter lamellar space
MSA.	-	Mucosa
RR.	-	Rod shaped receptor
SC.	-	Supporting cell
SMSA.	-	Sub mucosa
SR.	-	Spindle shaped receptor



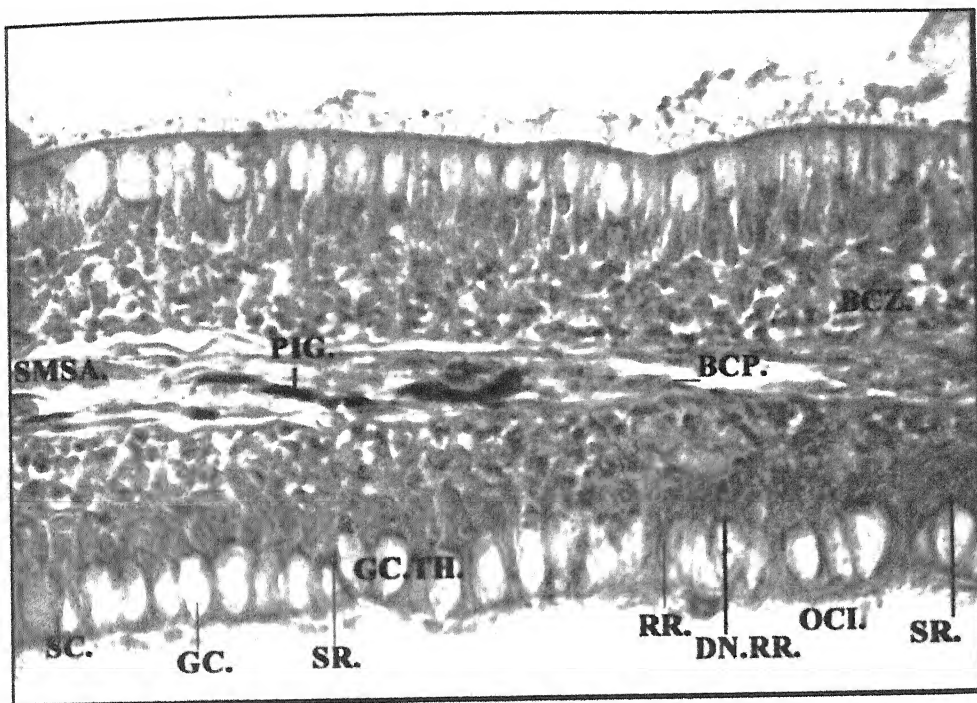


Plate.11

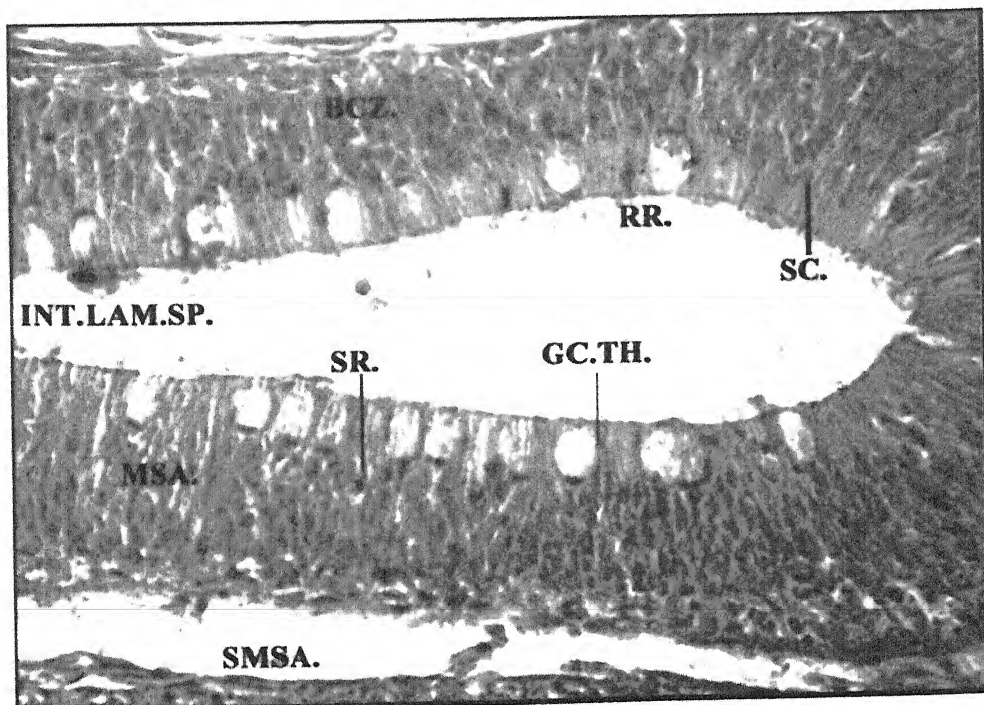


Plate.12

The rod shaped receptor cells (RR.) are commonly observed in the middle and distal regions of the olfactory epithelium of a lamella. Their dendrites (DN. RR.) are thick and rod shaped, extending either in between the theca of two marginal goblet cells or traversing singly or in groups through the empty theca of a goblet cell (Plates-9, 11, 12, 14, 16). The dendrite terminates distally in the form of expanded tip which bears minute cilia (OCI.) projecting in interlamellar space. The rod shaped receptor bears darkly staining narrow and elongated nucleus (NU.RR.). The axon is elongated, extending upto basal zone (BC.Z.) where they join to form folium olfactorium (FOL. OLF.).

The olfactory vesicles are observed in the terminal ends of the dendrite of rod and spindle shaped receptor cells in *C. carpio*. The spindle shaped receptor cells bear rounded vesicle while the terminal end of the dendrites of rod shaped receptor cells end terminally in the form of expanded tip forming olfactory vesicle of variable shapes. They are projected in the interlamellar spaces either by olfactory cilia or micro villi or both.

The presence of primary neuron in crypts and the projection of their cilia or protruding ends in theca (TH.) gives a shape of deeply embedded "Olfactory crypt" which can be commonly observed in the olfactory epithelium of *C. carpio*. (Plates-8, 10, 15, 16). The dendrites of rod shaped receptor cells also show their rare aggregation in the form of an "Olfactory crypt" on the uniform surface of the olfactory lamellae. The synaptic contacts in between any two receptor cells have not been observed any where in the olfactory epithelium of *C. carpio* and independant identity of each receptor cell is maintained. The

Plate-13: Magnified section of distal end of branch of *C. carpio* showing full formation of terminal bud possessing all cellular elements of the buds magnification 400 X.

TER. BUD

Terminal bud

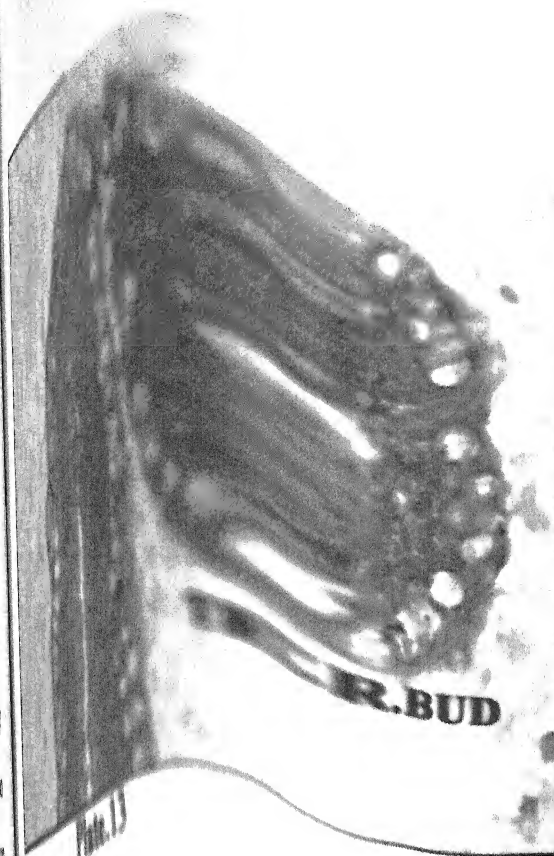


Plate 13

Plate-14: Magnified section of middle branch of *C. carpio* showing tremendous activity of polar cell which become grouped and reorganized and collectively burst out to form big saucer-like structure and also exerting pressure on underlying ones forcing them to migrate in any direction leading to the formation of surface elevation, crests of different shapes and sizes which possess the primary meristems in group or in solitary states. Magnification 700X.

GC.

Growing cell

PN.

Primary meristem

RR.

Round shaped receptor

SMSA.

Solid mass area

SR.

Specialized shaped receptor

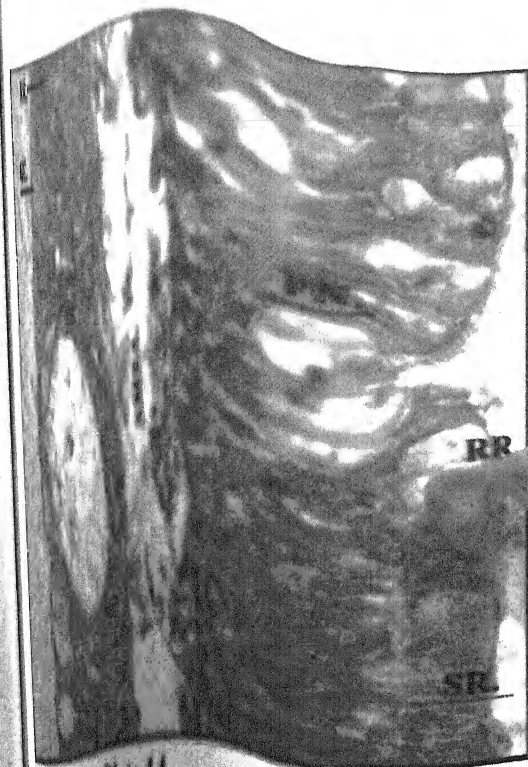


Plate 14

Plate-13 : Magnified section of distal end of lamella of *C. carpio* showing full formation of terminal bud possessing all cellular elements of the lamella. magnification 450 X.

TER. BUD - Terminal bud

Plate-14 : Magnified section of middle lamellae of *C. carpio* showing tremendous activity of goblet cell which become grouped and regrouped and collectively burst out to form big vacuole like structure and also exerting pressure on underlying zone forcing them to migrate in any direction leading to the formation of surface elevation, crypts of different shapes and sizes which possess the primary neurons in groups or in solitary states. Magnification 750X.

GC. - Goblet cell
PN. - Primary neuron
RR. - Rod shaped receptor
SMSA. - Sub mucosa
SR. - Spindle shaped receptor

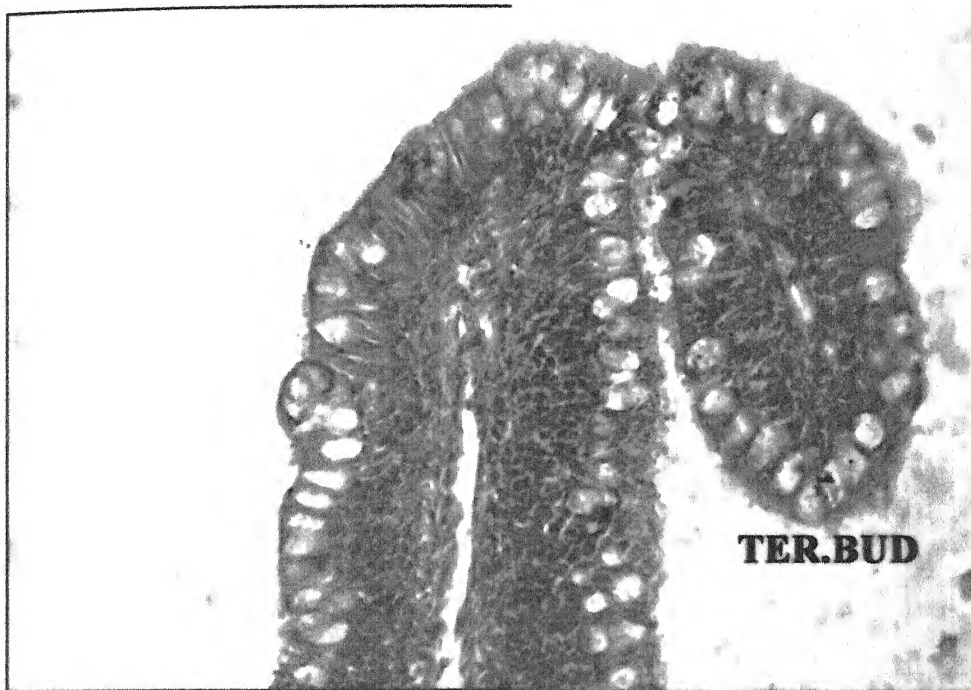


Plate.13

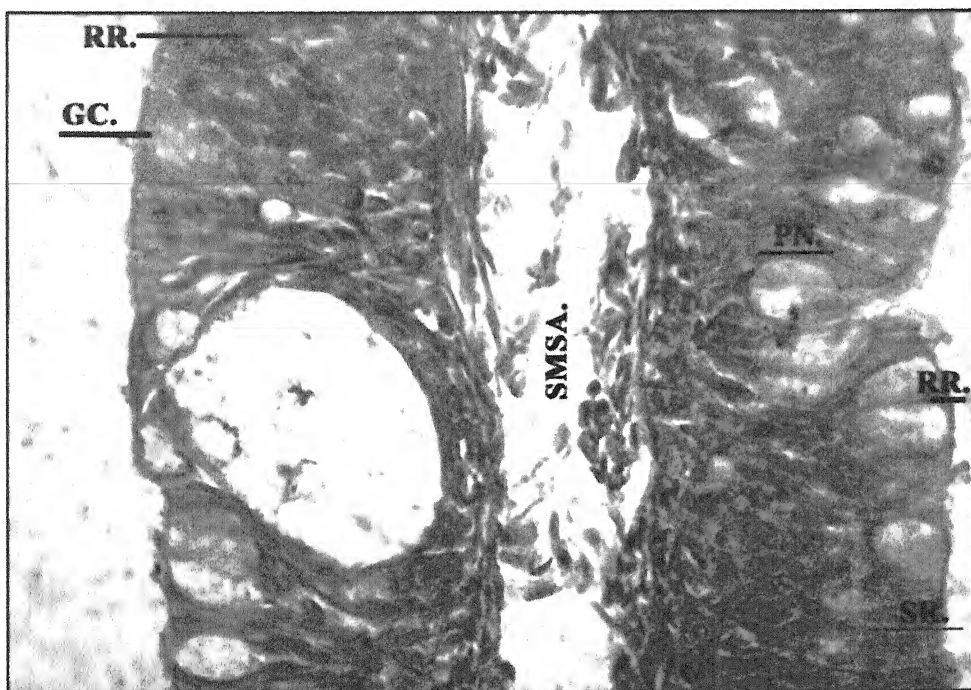


Plate.14

Plate-15 : Magnified section of hinder lamella of *C. carpio* depicting total breaking down of peripheral surface of mucosa in the form of crypts of different shapes and sizes accommodating large number of primary neurons which protrudes their dendritic end in their lumen. Rod and spindle shaped receptor cells are present on general surface, sending their dendrite or olfactory vesicle or olfactory cilia in the inter lamellar space spaces. Magnification 750 X.

Plate-16 : Magnified section of hinder lamella of *C. carpio* showing tremendous muciferous activity with the result of formation of different types of crypts. Due to the bursting of goblet cells there occurs migration of basal cells to the peripheral surface. Magnification 750 X.



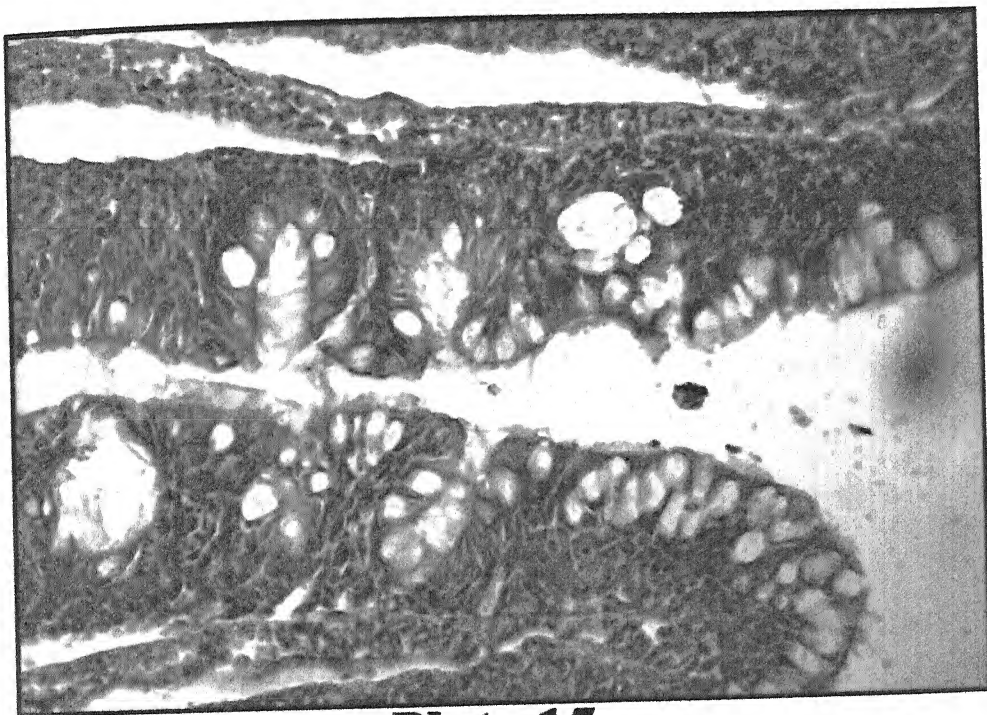


Plate.15

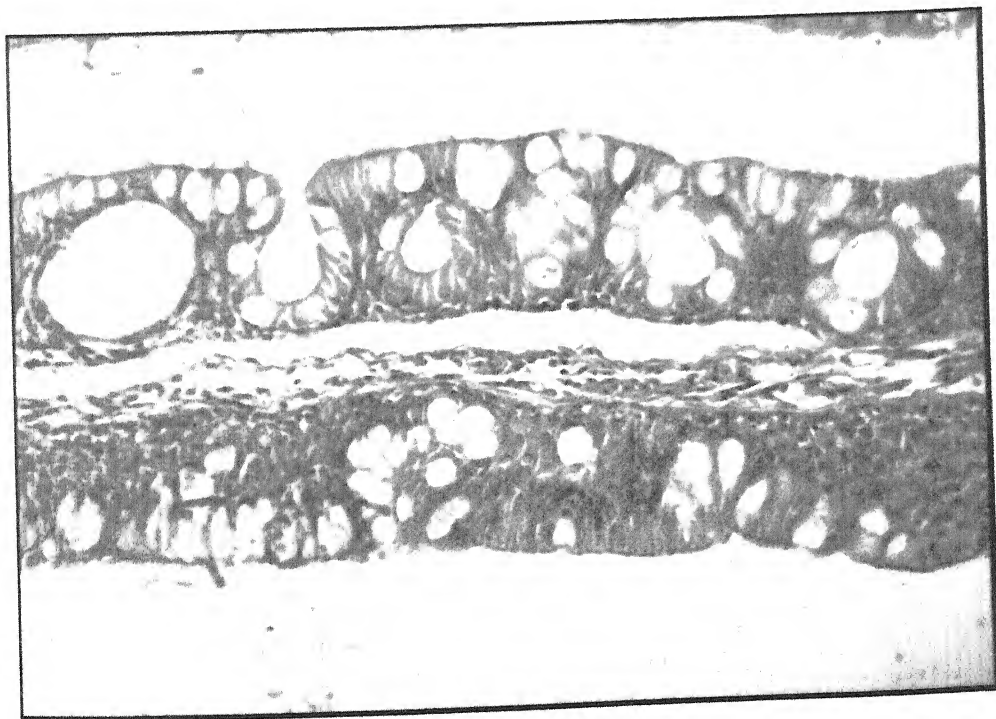


Plate.16

axons of all the receptor cells extend proximally and join folium olfactorium along basement membrane (Plate-10).

Goblet Cells :

These are the dominating cellular components of the olfactory epithelium of *C. carpio*. They can be easily distinguished into two types : (1) Marginal goblet (MG.) cells, (2) Migratory goblet cells (MIG.). The former are transformed by secondary supporting cells whereas later are the result of the specific basal cells lying in the proximal and intervening regions of the lamella adjacent to the raphe.

The marginal goblet cells are seen arranged serially throughout the peripheral surface of the lamella. They are provided with a cup shaped spacious theca (GC. TH.), which is filled with pale droplets of mucigen. The nuclear (NU.GC.) contents are very much compressed and pushed downwardly, leaving a small amount of darkly staining cytoplasm around the nucleus. The nucleus and cytoplasmic contents take a triangular shape in which nucleolus and chromatin material is not visible due to the high degree of compression. A stem like proximal limb connects the goblet cell with the basal zone (BC.Z.). The rod shaped receptors either lie in between the theca of these cells or traverse through the empty theca. The marginal goblet cells are produced continuously with the result of transformation of positively muciferous supporting cells with the age of the fish (Plates-8, 11, 12).

The migratory goblet cells (MIG.) originate from the muciferous basal cells which are concentrated in the proximal or intervening region of the lamellae adjacent to the raphe. They are shapeless and usually show rounded structure and remain in wandering tendency from deeper zones to peripheral zone of the olfactory epithelium.

Generally number of newly formed migratory goblet cells are grouped and fused in the form of complicated vacuole like structure, which gradually grows in size and ultimately burst out (GC. B.) from the peripheral surface of the lamella, discharging their mucous content in the inter lamellar space (Plates-9, 10, 14). This leads to the formation of crypts like formation which may be in the form of depression, flask, funnel and tubular deepening (Plates-15, 16). Due to the migratory process of these goblet cells, the olfactory epithelium is affected greatly causing the displacement of basal cells. This results the flow of basal cells in any direction which may lead to the formation of hillock elevation, straight projection, bifurcation and trifurcations from the general surface of the olfactory epithelium (Plates-13, 17, 18).

The grouping and fusion of the goblet cells at some places cause perfect interruption of the olfactory epithelium. Formation of crypts and microformations amount peculiar findings of this study as nowhere this phenomenon is noticed in the olfactory epithelium of the fishes studied so far.

Basal Cells :

The basal cells (BC.) can be distinguished in number of forms lying irregularly above basement membrane. The rounded forms of these cells are provided with darkly staining oval nucleus (NU.BC.) with a clear centrally placed nucleolus and uniformly distributed chromatin material in karyoplasm. The rounded basal cell can be observed anywhere in the olfactory epithelium. They are found distributed even in the extreme peripheral zone among the dendrites of receptors and distal limbs of the supporting cells. Their aggregation in groups can be commonly observed in the olfactory epithelium of C.

Plate-17 : Vertical section of trifid lamella of *C. carpio* showing all three lobes along with corner crypts, peripheral goblets, rod shaped receptors, spindle receptors and accumulation of primary neurons in corner and other crypts. Submucosa is sending its offshoots in all the three lobes making them a complete lamella form. Magnification 750X.

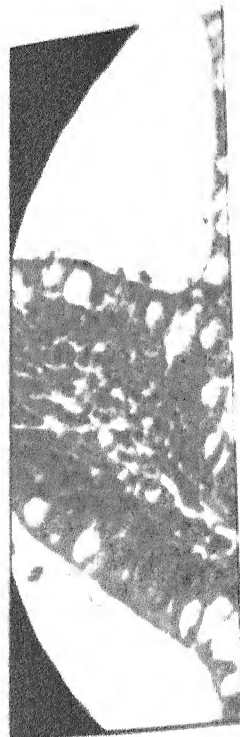


Plate-18 : Vertical section of terminal end of initial lamella of *C. carpio* showing bifurcation with no crypts but theca of goblet cell occupy the periphery of supporting zone which possess intervening ciliated, nonciliated supporting cells, rod and spindle shaped receptors. Submucosa is sending its offshoot in these two lobes. Magnification 450 X.





Plate.17

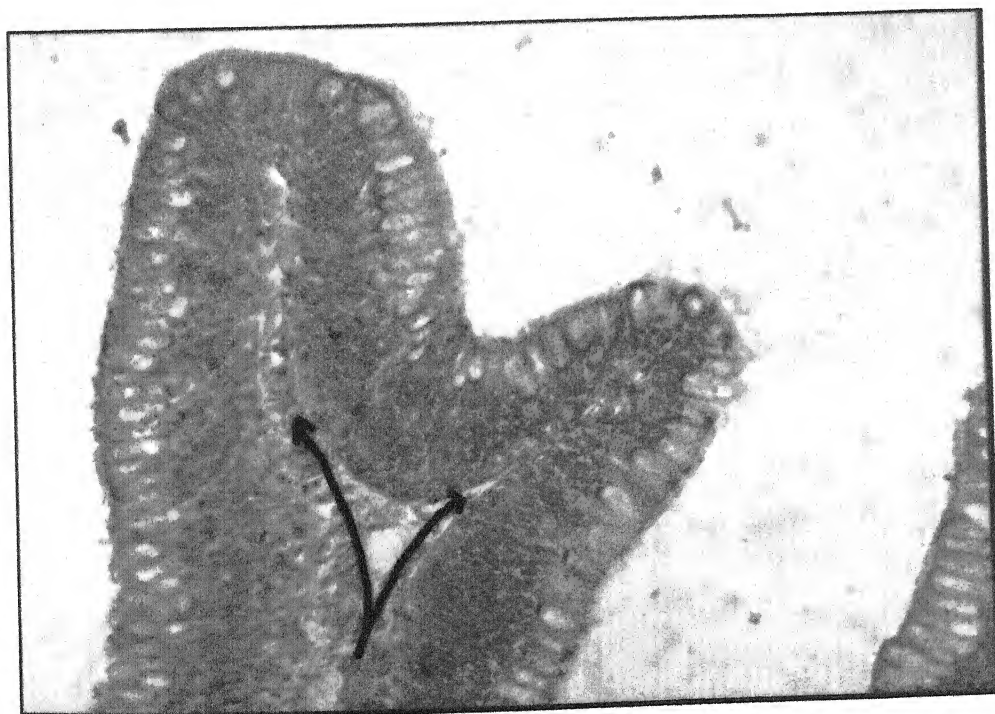


Plate.18

carpio which may be the initial preparation, leading to microformations in the surface of lamella. The larger form of the basal cell is observed uniformly distributed above the basement membrane in proximal and intervening regions of the olfactory epithelium of anterior lamellae. These are filled with highly muciferous cytoplasm which push the nuclear and cytoplasmic content to the extreme inner side, to give rise to migratory goblet cell. These basal cells are migratory form and show their shifting from proximal zone to the peripheral zone, giving rise to the crypts and microformations.

Fibroblast cells and irregular lymphoid cells can also be observed in the basal zone.

Central Core or submucosa :

The central core or submucosa (SMSA.) is lined on either side by the basement membrane. It is made up of dense collagen fibre connective tissues (CONN. IS. FIB.), which lies entangled in matrix. The presence of branched fibroblasts, histocytes, basal cells, and pigment cells are also noticed in the sub mucosa of *C. carpio*. Only folium olfactorium fibres run along the basement membrane and which join the nonmedullated nerve fibres (NMN.FIB.) at raphe. The blood supply is in form of finer blood capillaries (BCP.) and blood sinus passes through raphe. Thick collagen bundles are lying in the central core which entangles branched pigment cells. The connective tissue lying in submucosa is compact and no areolae are seen. It is in continuation with the submucosa of raphe. Thick collagen fibres provide strength to the lamellae forming a turgor like structure. The branching of submucosa is observed at the terminal bifurcation and

trifurcations, but in other places, it remains uniform and no offshoot formation is observed in other microformations.

Raphe :

The raphe is nonciliated, non sensory and median thickening of olfactory floor, which allow the attachment of all the lamellae on its either sides. It is composed of a spacious centralcore or submucosa with dense collagen fibres, basal cells, fibroblasts (FIB) and histocytes (HIC.) cells submerged in the thick matrix. Two rounded areolae (ARE.) and central blood sinus are seen in the submucosa of the raphe of *C. carpio*. Non-medullated nerve fibres (NMN.FIB.) extend along the basement membrane and join the folium olfactorium coming from lamellar regions. The mucosa (MSA.) of raphe is made up of cuboidal epithelium, consisting of cuboidal supporting cells, marginal goblet cells and basal cells. The margin of the raphe is totally occupied by the cupshaped theca of the goblet cells (GC.TH.), which is outwardly or distally covered by mucous sheath, secreted by these cells. Below the goblet cells lie one or two layers thick cuboidal cells whose distal processes extend upto the distal surface of the raphe. They have rounded darkly staining nuclei. The basal zone is three to five layers thick, lying in regular rows just above the basement membrane. The submucosa and mucosa of the raphe is in continuation with the lamellae. The nervous and nutritional supply in the lamellae is through the raphe.

Ecological Coefficient :-

It is calculated by two methods : first by taking the length as parameter of mesencephalon and telencephalon; second by measuring the areas of two retinae and both the rosettes. By comparing the former and

later parameters, the effectiveness of the olfactory and optic faculties can be assessed approximately from the anatomical point of view.

Five fishes of different sizes ranging from 113mm to 210mm are selected for calculating the ecological coefficient. It is observed that the length of the brain and the number of lamellae increases successively with the size of the fish.

The areas of two retinae and both the rosettes are measured by Teichmann (1954) method and further modified by Rahmani and Khan (1981). It is observed that the former ranges from 114.36mm² to 226.08mm² and that of later from 265.50mm² to 650.24mm² (Table-1). Though the areas of both the rosettes are found to be higher than the retinae but the value of later is of considerable significance and cannot be ignored. Considering the above values, it shows that *C. carpio* bears both olfactory and optic faculties better developed and, therefore, it can be identified as eye-nose fish and can be denominated as mesosmatic. In the natural habitat, the fish uses both the faculties with equal capability. This can increase the general efficiency of the fish and therefore, *C. carpio* is considered as most active exotic carp of fresh waters.

Route of water circulation through the olfactory chamber of *C. carpio* :

The posterior nasal opening is a wide aperture covering most of the area of the olfactory chamber and allowing exposure of the posterior part of the olfactory rosette to the external medium. Therefore, in *C. carpio* the olfactory epithelium remains in a constant touch with the water (similar to the gills).

In addition to it, the forward movement of the fish and synchronously the unidirectional beating of the cilia of the olfactory

epithelium, causes the entry of water current through anterior nasal opening to the central part of the outer concave surface of rosette. From here it is directed to the central and peripheral channels, leading to its ultimate expulsion from the posterior nasal opening. The forwardly directed nasal flap deflects the water current to the anterior nasal opening. During the course of circulation, water passes through the interlamellar spaces and lamellae are bathed properly.

The fish in motionless condition enjoys a constant contact of the olfactory lamellae with water through the posterior nasal opening but during forward movement, the current of water enters through the anterior nasal opening and is virtually expelled out from the posterior.

The olfactory epithelium of *C. carpio* is intensively mucous secretory and it is observed that foreign materials are trapped from the water current by the mucous at certain places in the inter lamellar spaces. This may be a device for removing the unwanted foreign material from the water circulating over the olfactory rosette through the outgoing water current. This device can be compared with mucous secretion of the nasal epithelium of mammals which makes the air dust free before its intake in the alveoli.

Table-7 : Ecological coefficient of *C. carpio*

Sl. No.	Total length (mm)	Number of lamellae Rosette		Total length of brain (mm)	Length of mesencephalon (mm)	Length of telencephalon (mm)	Ecological coefficient (through lobes of brain) Length of telecephalon x 100 Length of mesencephalo	Retinal area of both eyes (mm ²)	Olfactory area of both rosette (mm ²)	Ecological coefficient (through area) Olfactory area x 100 Retinal area
		Right	Left							
1.	113	23	24	5.70	2.29	1.58	68.99	114.36	265.50	232.16
2.	145	30	30	8.19	3.27	1.98	60.55	127.16	280.24	220.38
3.	165	32	32	8.77	3.74	2.57	68.71	156.00	585.62	245.61
4.	185	34	33	10.53	3.86	2.57	66.58	226.08	601.44	266.02
5.	210	36	36	11.21	4.09	2.92	71.39	226.08	650.24	287.61

Histochemical Observations of *Cyprinus carpio*

Histochemical descriptions are meant for explaining correct morphological concepts of biological systems. In the present study, attempt has been made to demonstrate the histochemical localization of acid phosphatase, alkaline phosphatase, lipid, glycogen and acid mucopoly sachharides in the olfactory epithelium of *Cyprinus carpio*.

Acid Phosphatase :

The enzyme histochemical reactions have been treated as a link between morphology and biochemistry. An attempt has been made in the present study for the histochemical demonstration of acid phosphatase in the olfactory epithelium of *Cyprinus carpio*.

Acid phosphatase has been regarded as marker enzyme for lysosome. Recent evidence shows that acid phosphatase has not been restricted to lysosomal fraction but is also formed in golgi cisternae, and specialized region of endoplasmic reticulum as "GERL" (Farquhar *et al.* 1974).

In the acid phosphatase preparation of olfactory epithelium of *Cyprinus carpio*, all the cellular components are showing positive reactions. The localization of acid phosphatase in the olfactory epithelium was considered as one of the confirmatory indices for the identification of neurosecretory functions (Barymann and Zelforseh, 1949). Baronyi (1966) reported that acid phosphatase plays a role in the process of catabolism.

The synaptic junction shows low intensity reaction for acid phosphatase. This may be attributed to the fact, that, the animals

were collected in winter when most of the metabolic activities slows down.

The axon shows moderate acid phosphatase activity in the olfactory epithelium of *Cyprinus carpio*. This may be due to uncoupling of phosphorylation followed by cells during the olfaction.

Increase in the activity of acid phosphatase in the primary neurons or receptors cells, spindle and rod shaped receptor cells, secondary neurons, columnar supporting cells and goblet cells of olfactory epithelium is due to more hydrolytic enzymes which are concerned with the lysosomes. Increased activity of acid phosphatase in spindle shaped neurons of *Cyprinus carpio* is correlated with increased catabolic activity and their sudden increase in the activity suggests a metabolic readjustment.

The lysosomal enzymes are associated with degradative processes and their higher activities are often correlated with greater turnover of molecules (Allison, 1953) (Table-1)

Alkaline Phosphatase :

Alkaline phosphatase activity in *C. carpio* occur in the basal cells in a relatively high degree. The intense activity is seen in the aggregation of these cells at interlamellar levels and at the base of the olfactory epithelium in the distal region of the growing lamellae.

The spindle shaped neurons displayed a moderate reaction along the nuclear membrane while primary neurons rod shaped receptors and synaptic junction are also stained brownish black, indicating the low concentration or mild enzyme activity (Table-2). The columnar supporting cells and goblet cells show negative response with stain (Table-2).

The difference in the alkaline phosphatase level in the primary neurons, spindle and rod shaped receptor cells, synaptic junction, columnar supporting cells, basal cells and goblet cells may be related to the differential rate of substrate hydrolysis and transfer of metabolites across the olfaction site. High activity in the basal cell may be linked to considerable demand of the metabolites to mobilize and transfer large amount of energy rich precursors. (Table-2). The low concentration of the enzyme may be due to lesser demand and transfer of metabolites.

Glycogen :

Metabolic contribution of carbohydrate metabolism is often reflected in alterations in the glycogen, the major carbohydrate reserve. Hence the study is initiated to note the variation in the glycogen content during olfaction.

Occurrence of glycogen in the olfactory epithelia show a seat of intense biological activity and glycogen as a readily available source of energy will be required to support the sense of olfaction.

For localization of glycogen in *C. carpio* the rosette were fixed in appropriate fixative. 8µm thick section were processed and Best Caramine technique were used for demonstration.

The observations (Table-3) reveal that heavy deposits of glycogen occur in the columnar supporting cells around the distal limb and basal cell. Heavy reserves also occur in the goblet cells. It is also found in the present study that synaptic junction showed moderate deposition which denotes that glycogen were utilized rapidly for the supply of energy in sense of olfaction.

The decline in the glycogen content is greater in the primary neurons, spindle and rod shaped receptor cells. This could be due to their utilization in olfaction, fuel energy production and their probable contribution to the protein build up in the neurons. According to Tate and Winter (1962) the depletion in the glycogen is due to its transformation into protein and lipid.

Heavy deposits of glycogen in the columnar cells, basal cells and goblet cells indicate utilization of this polysachharides during olfaction (Table -3).

Acid Mucopolysaccharides :

The mucous secreting goblet cells of olfactory epithelia of *C. carpio* retain a brilliant bluish green stain with Alcian blue which demonstrates the intense deposition of mucopolysaccharides whereas in the distal limbs of the columnar supporting cells and in some basal cells mild stain is seen. Except these components of the olfactory epithelia, non of the other cellular components showed any reaction with the Alcian blue. So it has been assumed that the goblet cells are reservoir of mucopolysaccharides whereas its activity in columnar supporting cell and some basal cells indicate that these are in muciferous position and ultimately convert into goblet cells to compensate the enhanced mucous activity in the olfactory epithelium (Table 4).

The excessive mucous is meant for lubricating the delicate olfactory epithelial surface as well as to protect it with the damaging effect of constantly circulating water current with different degree of striking pressure. It is also meant for entangling foreign bodies and

isolating them from circulating water current and ultimately removing such unwanted mass through outgoing water current.

Acid mucopolysaccharides are complex carbohydrates characterised by the presence of a hyaluronic acid along with a N-acetyl hexosamine, stable and resistant to chemical hydrolysis and therefore, they are found at places where strength and chemical resistance is required (Sinha *et al.*, 1978).

Hyaluronic acid, a biologically important acid mucopolysaccharide found in many animal tissues, act as a barrier to fluid diffusion and prevent the leakage of material and impulse across the cell membrane. The acid mucopolysaccharides is also utilised rapidly for the supply of energy. The functional role of acid mucopolysaccharides seems to aid in binding with calcium ion in *C. carpio* and fulfilling the requirement of energy consumed during receptory process through odorant.

Thus, acid mucopolysaccharides detected in olfactory epithelia play an important role in olfaction by acting as selective ion barrier and by initiating impulse due to depolarization of the membrane.

Lipids :

Lipids are present in high concentrations in the distal tips of columnar supporting cells of olfactory epithelium *C. carpio*. Comparatively moderate quantities also occur in the mucin granules of goblet cells, cytoplasm around nuclei of primary neurons, dendrites of primary neurons, synaptic junctions between primary and secondary neurons and proximal limb of columnar supporting cells. Mild concentration of lipids are also present in the nuclear

membranes of various cell types, cytoplasm of basal cells, cilia of columnar supporting cells and axons of secondary neurons (Table-5).

Meta cromasia :

Metacromasia in *C. carpio* is demonstrated in the proximal part of the dendrites of primary neurons, synaptic junction between primary and secondary neurons and the cytoplasm around the nuclei of primary and secondary neurons. A comparative milder reaction is observed in the goblet cell, axon of secondary neuron, proximal limb of columnar supporting cell and basal cell (Table-6).

Table-1 : Showing Histochemical demonstration of Acid phosphatase employed by Pearse 1968, and the reaction obtained in various cellular components of olfactory epithelium of *Cyprinus carpio*.

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
Acid Phosphatase	Cryostat	Modified Lead nitrate method Processed as recommended by TAKEUCHI and TANOUE as given by Pearse, 1968.	(i) Primary neurons	+++	+++ = High activity
			(ii) Spindle shaped receptors cells	+++	++ = Moderate activity
			(iii) Rod shaped receptors cells	++	+ = Low activity
			(iv) Synaptic junction	+	- = Absence of any activity
			(v) Columnar supporting cell	+++	
			(vi) Basal cell	+++	
			(vii) Goblet cell	++	

Table-2 : Showing the demonstration of **Alkaline phosphatase** activity employed by Pearse 1968, and the reaction obtained in various cellular components of olfactory epithelium of *Cyprinus carpio*.

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
Alkaline Phosphatase	Cryostat	Calcium-Cobalt method [after GOMORI, as given by Pearse 1968]	(i) Primary neurons	+	+++ = High activity
			(ii) Spindle shaped receptors cells	++	++ = Moderate activity
			(iii) Rod shaped receptors cells	+	+ = Low activity
			(iv) Synaptic junction	+	- = Absence of any activity
			(v) Columnar supporting cell	-	
			(vi) Basal cell	+++	
			(vii) Goblet cell	-	

Table-3 : Showing the histochemical localization of **Glycogen** in olfactory epithelium of *Cyprinus carpio*.

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
Glycogen	Microtomy 8µm	Periodic Acid- Schiff technique and Best Carmine stain	(i) Primary neurons	+	+++ = High activity
			(ii) Spindle shaped receptors cells	+	++ = Moderate activity
			(iii) Rod shaped receptors cells	+	+ = Low activity
			(iv) Synaptic junction	++	- = Absence of any activity
			(v) Columnar supporting cell	+++ (Distal limb)	
			(vi) Basal cell	+++	
			(vii) Goblet cell	+++	

Table-4 : Showing the histochemical localization of **Acid mucopolysaccharide** in the various cellular components of olfactory epithelium of *Cyprinus carpio* (employed by Pearse, 1968).

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
Acid mucopolysaccharides	Microtomy (8µm)	Alcianblue method [after STEEDMAN, vide PEARSE, 1968]. Deposition showed the bluish green stain with Alcian Blue	(i) Primary neurons	-	+++ = High activity
			(ii) Spindle shaped receptors cells	-	++ = Moderate activity
			(iii) Rod shaped receptors cells	-	+ = Low activity
			(iv) Synaptic junction	-	- = Absence of any activity
			(v) Columnar supporting cell	+	
			(vi) Basal cell	+	
			(vii) Goblet cell	+++	

Table-5 : Showing histochemical technique for demonstration of **lipid** in the olfactory epithelium of *Cyprinus carpio*

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
LIPID	Microtomy (8µm) (Temporary mount)	Sudan Black B method (after Mc MANUS Vide PEARSE, 1968)	(i) Primary neurons	++ (Cytoplasm around nucleus and dendrites)	+++ = High activity
			(ii) Spindle shaped receptors cells	+ (axon)	++ = Moderate activity
			(iii) Rod shaped receptors cells	+ (axon)	+ = Low activity
			(iv) Synaptic junction	++	- = Absence of any activity
			(v) Columnar supporting cell	(a) ++ (distal limb) (b) ++ (proximal limb) (c) + (cilia)	
			(vi) Basal cell	+ (cytoplasm)	
			(vii) Goblet cell	++	

Table-6 : Showing histochemical localization of **metacromasia** in the olfactory epithelium of *Cyprinus carpio*.

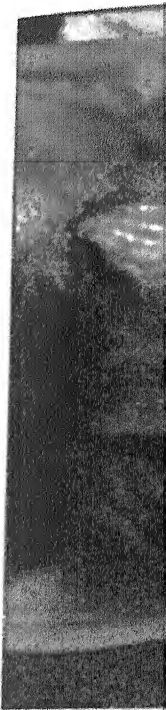
ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
Metacromasia	Microtomy (8µm)	Toulidine Blue method (after KRAMER and WINDRUM, as given by PEARSE 1968)	(i) Primary neurons	+++ (Proximal part of dendrites)	+++ = High activity
			(ii) Spindle shaped receptors cells	++ (cytoplasm around nucleus) + (axon)	++ = Moderate activity + = Low activity
			(iii) Rod shaped receptors cells	++ (cytoplasm around nucleus) + (axon)	- = Absence of any activity
			(iv) Synaptic junction	++	
			(v) Columnar supporting cell	+ (proximal limb)	
			(vi) Basal cell	+ (proximal limb)	
			(vii) Goblet cell	+	

Plate-1 : Lateral view of head of *B. bagarius*

ANT. NAS. OP.	-	Anterior nasal opening
Eye	-	Eye
NAS.FLAP	-	Nasal flap
POST. NAS.OP.	-	Posterior nasal opening

Plate-2 : Dissection of the head of *B. bagarius* from lateral side to show rosette in situ

ROS.	-	Rosette
RPH.	-	Raphe
VEN.LAT.ACC.	-	Ventro lateral accessory
nasal sac		
NAS.SAC		



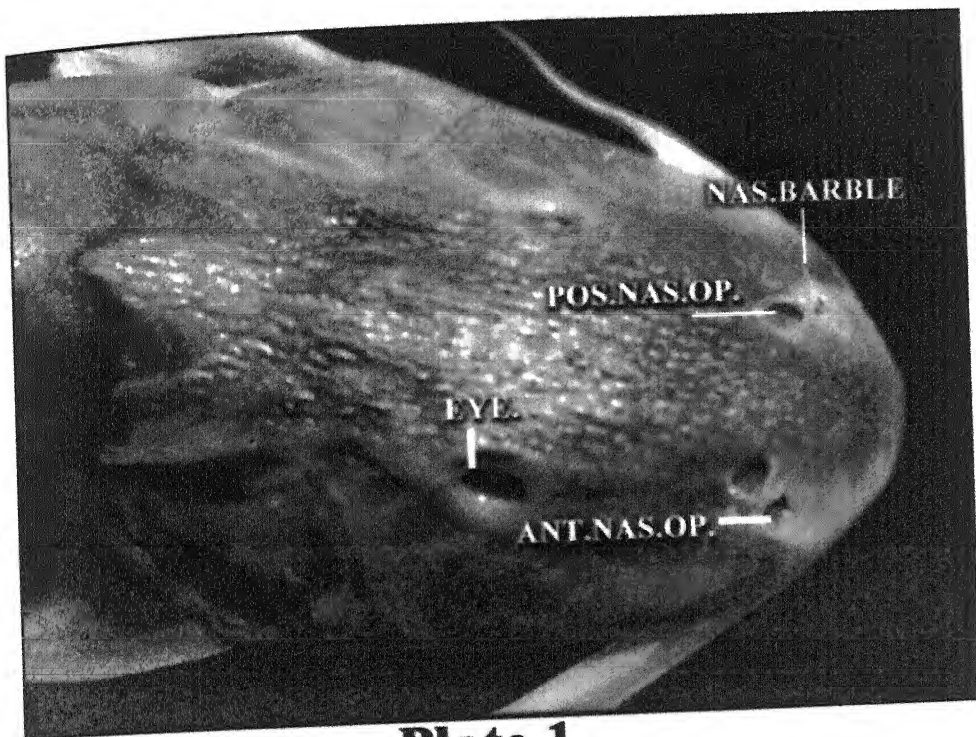


Plate.1

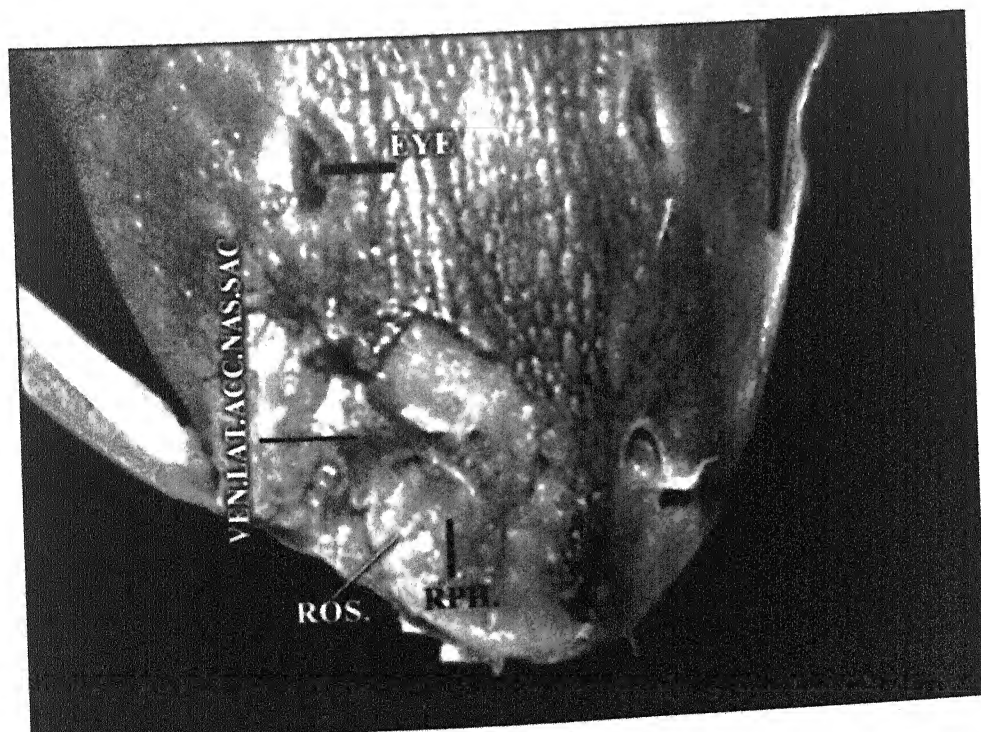


Plate.2

Histological Observations of The Olfactory Organ of *Bagarius bagarius*

B. bagarius bears a pair of olfactory chambers on the dorsal surface of the head, lying close to the snout and away from the eye orbit (Fig.-1A, Plates-1,2). They are ventilated out side by a pair of openings which can be named as anterior and posterior nasal openings which can be named as anterior and oposterior nasal openings (ANT. NAS. OP. and POST. NAS. OP.) with regards to their respective position. The anterior nasal opening is tubular over hanging on the upper lip, while, posterior is valvular and flush with surface of the head (Figs.-1A, Plate-1, 2). The later is in the form of an oblique furrow surrounded by the loose cresentric area of the integument and is made of anterior and posterior lips of the skin (ANT. LIP and POST. LIP, Fig.-38D). The former gets expanded over the later giving a shape of valve to the posterior nasal opening which regulates the entry and exit of water current through the olfactory chamber (OLF.CHAM.). Anterior to the posterior nasal opening lies a nasal barble (NAS. BAR., Fig. 1A, Plate-1), whose movement causes effective variation in the volume of the olfactory chambers. It is (olfactory chamber) enormously developed with a leaf shaped appearance, accommodating the rosette (ROS.) and the accessory nasal sac (VEN.LAT.ACC.NAS.SAC., Plate-2). The olfactory rosette is leaf shaped elongated structure having anterior broad and posterior narrow ends (Fig.-1B) It consists of thick olfactory epithelium and give rise to numerous lamellae (LAM.), attached on either sides of the raphe (RPH., Fig.-1B, C) The rosette is almost flat and is attached with the floor of the olfactory chamber by

- Fig.1 A Diagram of the lateral view of the head of *B. bagarius*.
- Fig. 1 B Diagramatic sketch of the right rosette of *B. bagarius*.
- Fig.1 C A set of 1 - 32 lamellae from one half of the rosette of *B. bagarius*.

ANT. NAS. OP	:	Anterior national opening
ANT. NAS. TUBE	:	Anterior nasal tube
LAM.	:	Lamellae
LING. P.	:	Linguiform process
NAS. BARBLE	:	Nasal barble
OLF. CHAM	:	Olfactory Chamber
POS. NAS.OP	:	Posterior nasal opening
ROS.	:	Rosette.
RPH	:	Raphe

NAS BARBLE
POS NAS OP
OLF CHAM
ANT NAS OP

MAX BARBLE



B



26

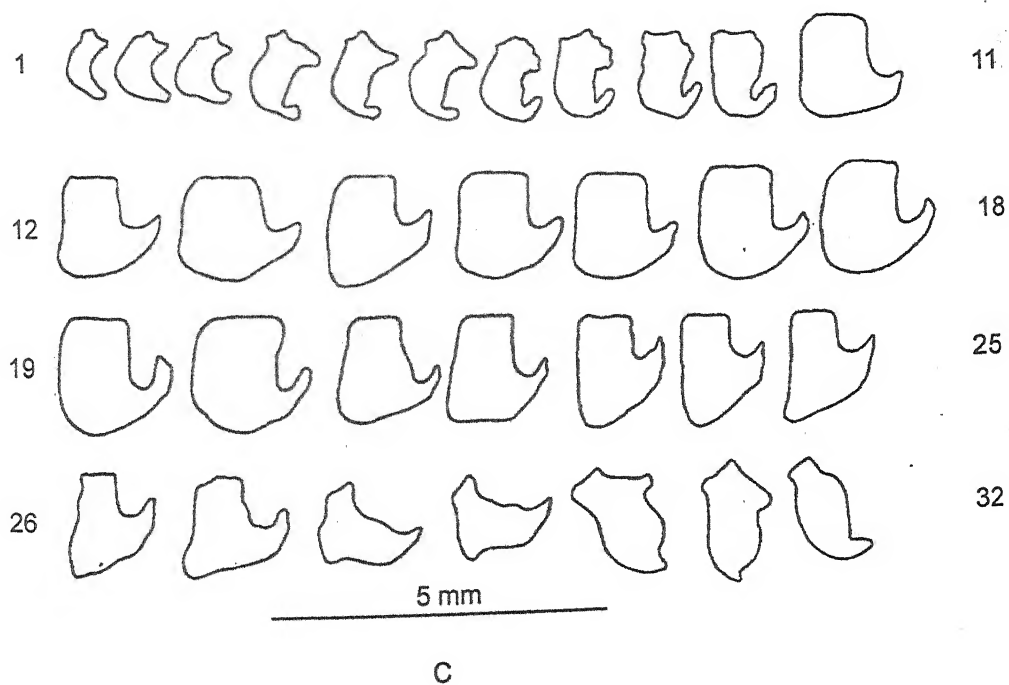
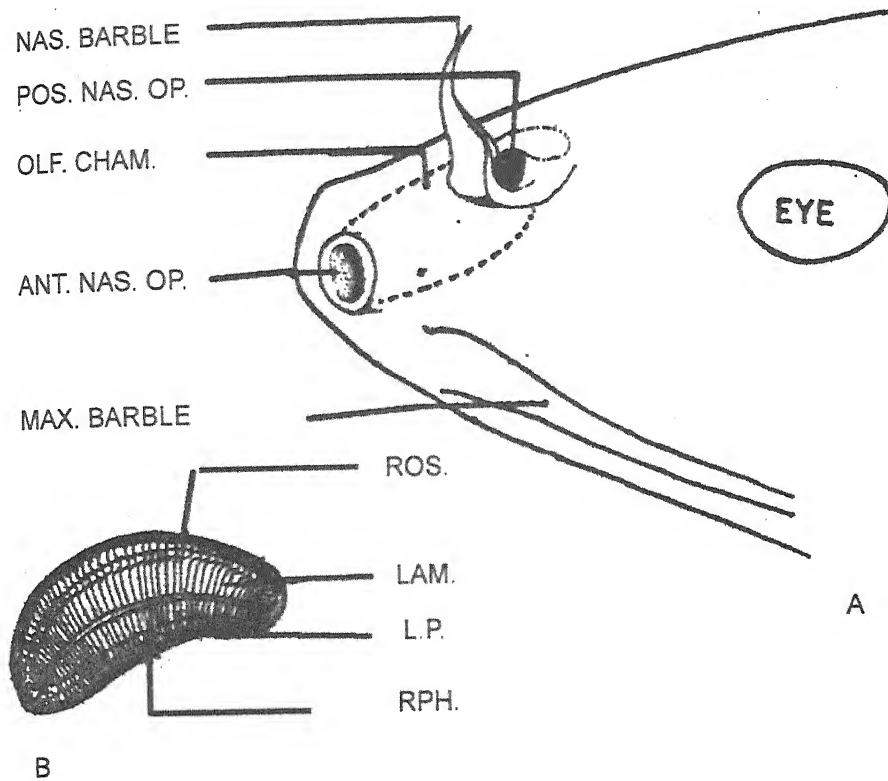


Fig. - 1

Fig.2 : Diagram of the dissection of the head of *B. bagarius* from dorsal side to show the relationship of brain with the rosette.

CE.	:	Cerebellum
EY.	:	Eye
OLF. BL.	:	Olfactory bulb
OLF. LO.	:	Olfactory lobe
OLF. TR.	:	Olfactory tract
ROS.	:	Rosette
VEN. LAT.	:	Ventro lateral Accessory
ACC. SAC		nasal sacs

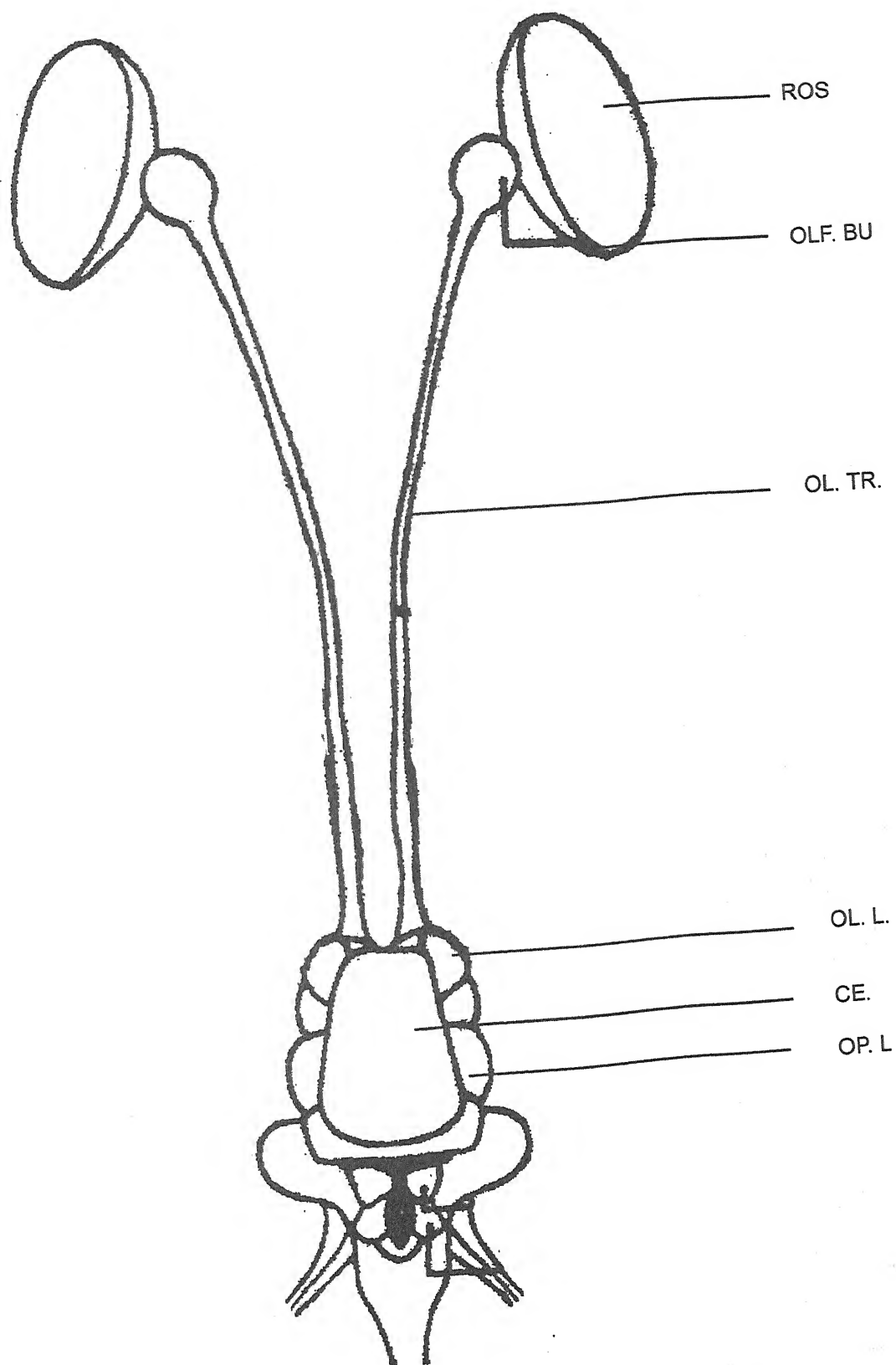


Fig. - 2

fibrous connective tissue. The peripheral and central channels are present in each halves of the rosette and continuous antero-posteriorly ascending series of linguiform process (LING.P.) stand as partition in between them. The posterior extremity of the olfactory rosette is narrow and lamellaeless (LAM.LESS) where the accessory sac opens by an independent aperture.

The lamellae (LAM.) of *B. bagarius* are short and broad which are attached proximally with the raphe and distally with the wall of the olfactory chamber (W.OLC.CHAM., Figs.-1B, Plates-3, 4, 5). Their dorsal surface is free and maintains inter-lamellar space (INT. LAM. SP., Plates-3,4,5) in between them. The dorsal medial surface of each lamella is projected out in the form of a thumb like linguiform process, arranged in an anteroposteriorly ascending manner, which forms curtain like separation in the centre of each half of the rosette.

After removing the median ethmoid, lateral ethmoid and frontals from the dorsal side of the head, the brain and its relation to the olfactory rosette become clearly exposed. The olfactory bulbs (OLF. BL.) are situated close to the postero-ventral surface of the rosette and receive the nerve fibres from each lamella. The olfactory bulbs are anteriorly broad and become narrow posteriorly which are joined with telencephalon by thick olfactory tracts (OLF. TR., Fig.-2). The olfactory lobe (OLF.L.) is better developed as compared to the optic lobe (OP.L.). The size of brain and its lobes are found increasing successively with respect to the size of the fish (Table-7).

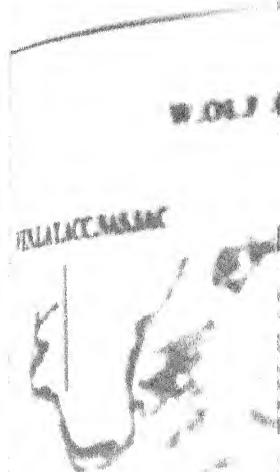
Olfactory epithelium forms the outlining of olfactory chamber and is thrown into the number of lamellae which are attached on either sides of the raphe (RPH., Plates-3, 4). It is a median antero-

Plate-3 : Horizontal section rosette of *B. bagarius* showing lamellar arrangement with respect to aphe and olfactory chamber. The ventro lateral accessory nasal sac is also shown in relation to rosette. Magnification 50X.

DIS.E. LAM.	-	Distal end of lamella
INT.LAM.SP.	-	Inter lamellar space
PRO.E.LAM.	-	Proximal end of lamella
RPH.	-	Raphe
W.OLF.CHAM.	-	Wall of olfactory chamber
VEN.LAT.ACC.NAS.SAC	-	Ventro lateral accessory nasal sac

Plate-4 : Magnified section of rosette of *B. bagarius* showing supply of connective tissue, blood capillaries, nervous elements from submucosa of raphe to submucosa of lamellae. Magnification 100X.

BCP.	-	Blood capillaries
CONN. TIS.FIB.	-	Connective tissue fibres
HIN.LAM	-	Hinder lamella
INT.LAM.SP.	-	Inter lamellar space
INI.LAM.	-	Initial lamella
MID.LAM.	-	Middle lamella
MIN.LAM.	-	Minor lamella
MSA.	-	Mucosa
NMN.FIB.	-	Non medullated nerve fibre
RPH.	-	Raphe



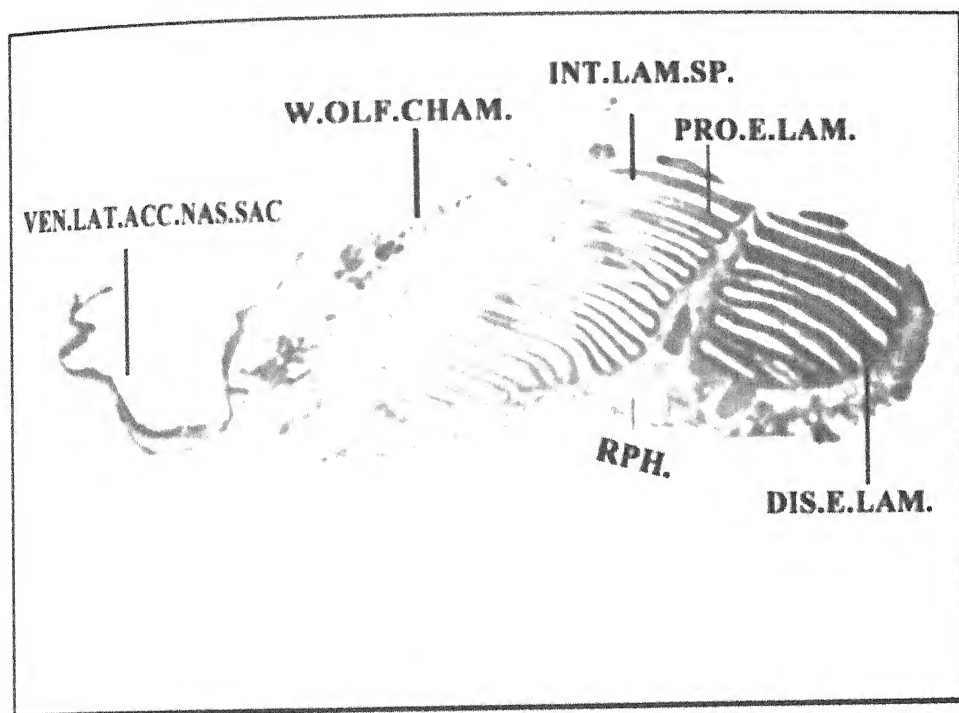


Plate.3

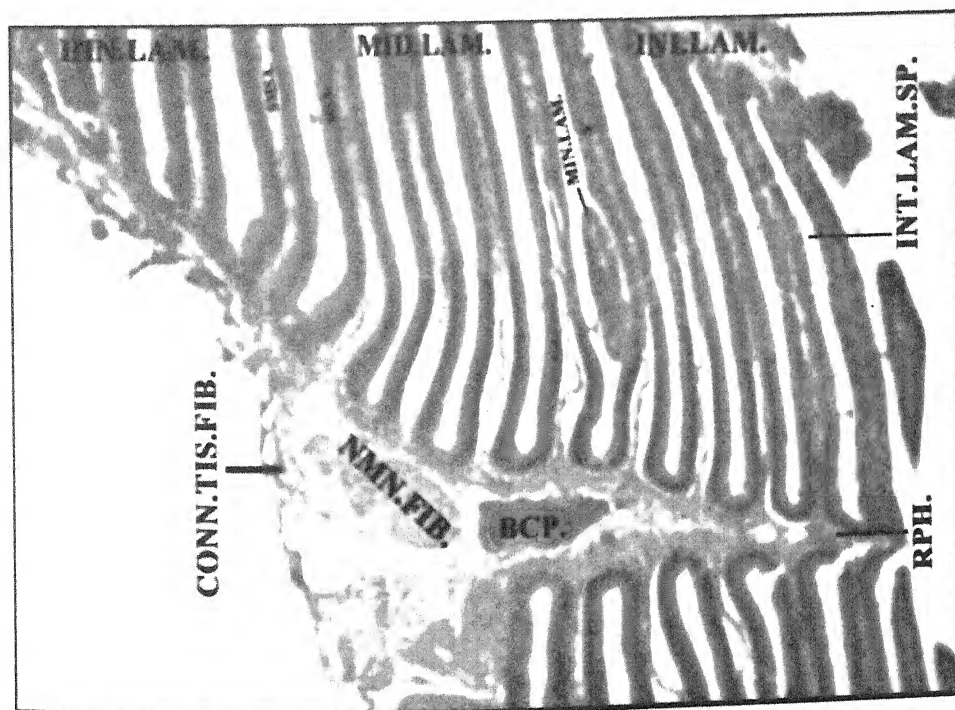


Plate.4

Plate-5 : Horizontal section of rosette of *B. bagarius* showing one half of lamellar arrangement with olfactory chamber of raphe along with demarcation of distal, middle and proximal end of lamella. Magnification 100 X.

DIS.E. LAM.	-	Distal end of lamella
INT.LAM.SP.	-	Inter lamellar space
MSA.	-	Mucosa
PRO.E.LAM.	-	Proximal end of lamella
RPH.	-	Raphe
SMSA.	-	Sub mucosa
W.OLF.CHAM.	-	Wall of olfactory chamber

Plate-6 : Magnified section of proximal end of initial lamella of *B. bagarius* showing compact submucosa, mucosa, thick basal zone, ciliated and nonciliated supporting cells, independent spindle shaped receptor and primary neurons. Synaptic contact in between these receptors is occasionally visible which is indicated by arrow. Magnification 450 X.

BCP.	-	Blood capillaries
CONN. TIS.FIB.	-	Connective tissue fibres
FOL.OLF.	-	Folium olfactorium
PN.	-	Primary neuron
SC.	-	Supporting cell
SR.	-	Spindle shaped receptor
SYN.	-	Synapse

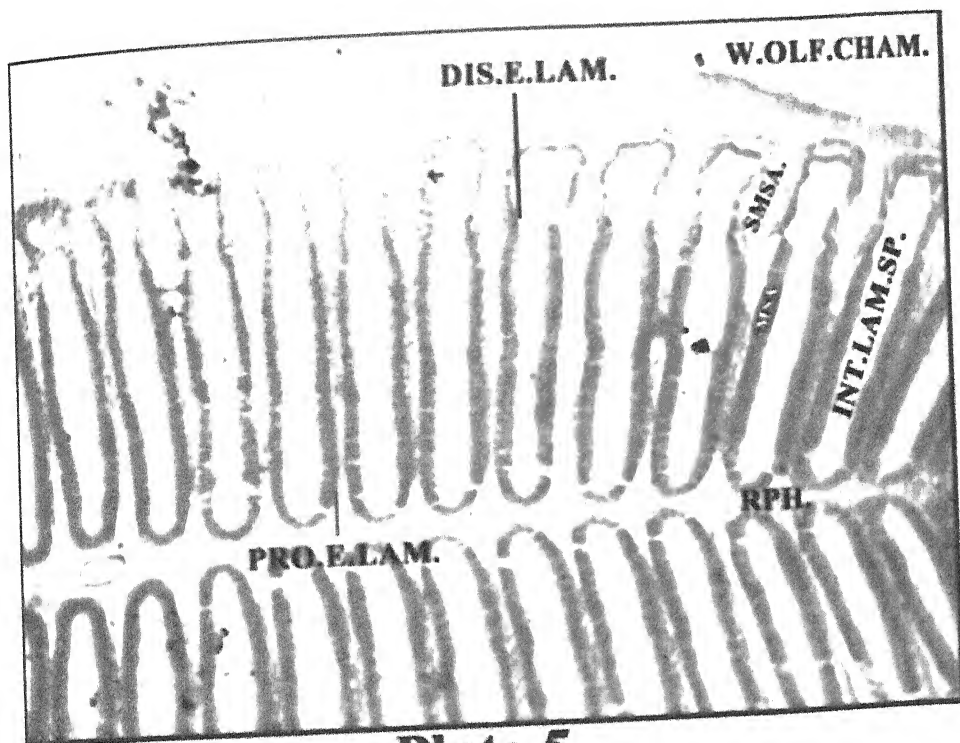


Plate.5

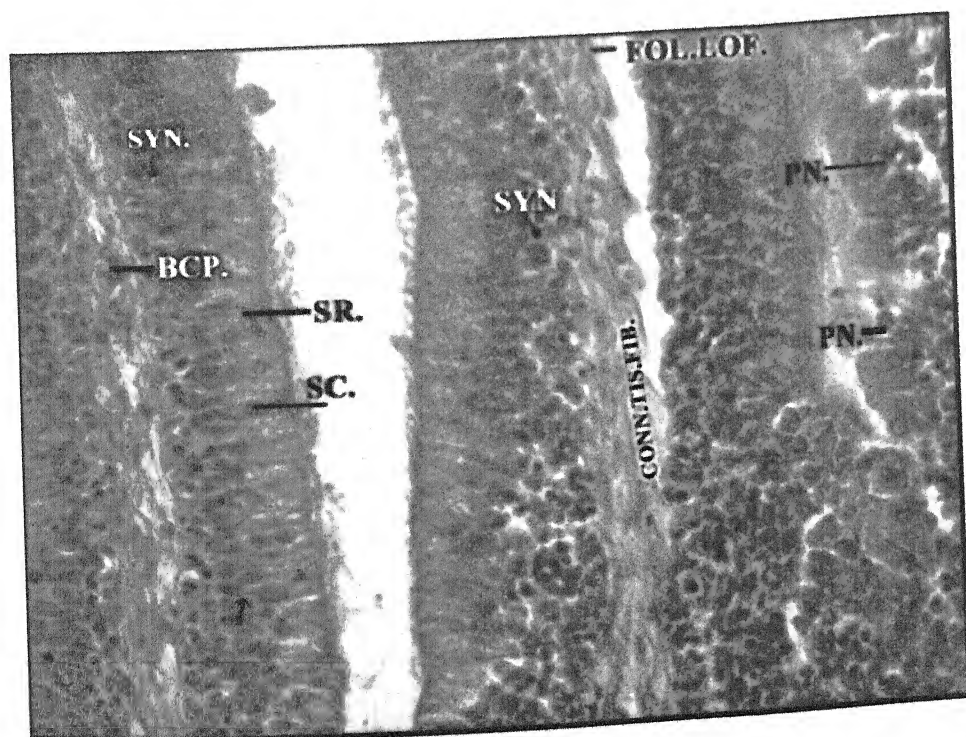
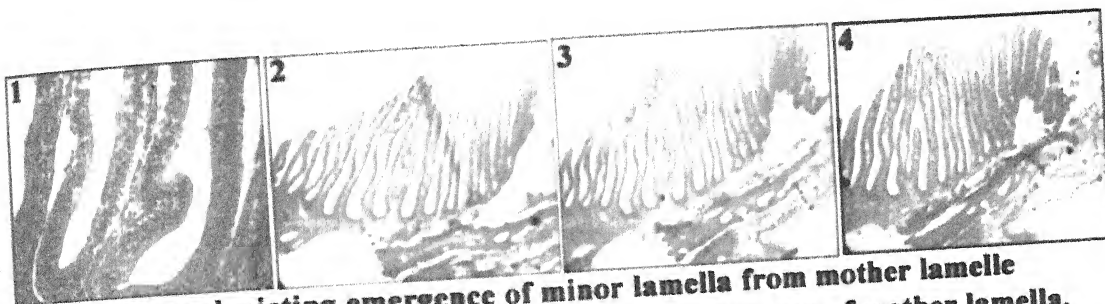


Plate.6

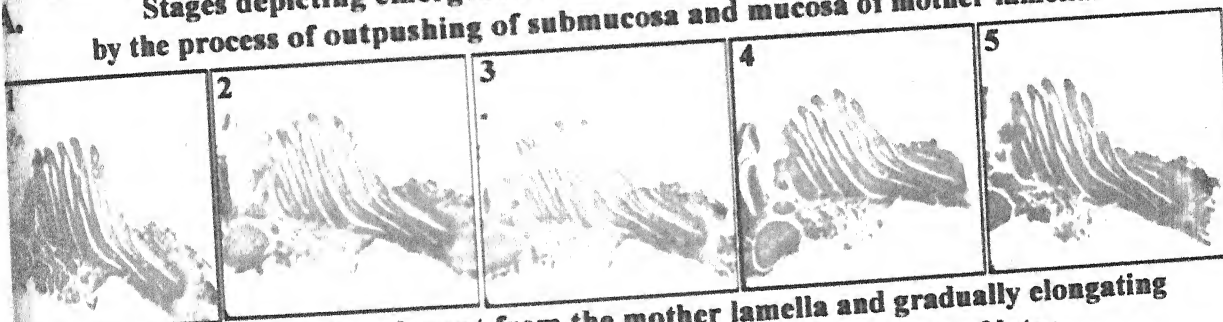
posterior thickening of the olfactory epithelium, dividing the rosette in two clear halves. The olfactory lamellae are encapsulated by the ventro-lateral expansion of the olfactory epithelium (W. OLF. CHAM, Plates-3,4,5) but their dorsal and outer ends remain free, maintaining interlamellar space (INT. LAM. SP., Plates.-3,4,5) inbetween them. Each lamella is made up of central core or submucosa (SMSA.) which is an extension of the tissue underlying the ventral wall of the olfactory chamber. The central core or submucosa is lined by the cellular component of the olfactory epithelium or mucosa (MSA.) on either sides so that a lamella is virtually supported by two layers of sensory epithelium (Plates-4,5,12) From histological point of view, all the lamellae of a rosette can be divided in three groups : initial; middle and hinder (Plate-4). The cellular organization of these three division of lamellae varies greatly.

The initial lamellae are having compact cellular organization. The central core or submucosa and epithelial cellular lining are well built, giving the impression of youngest lamellae of the rosette. They bear short, narrow structure with mucous secretory goblet cell on the extreme tip. Submucosa is comparatively narrow having rich blood and connective tissue supply (Plates-6,12)

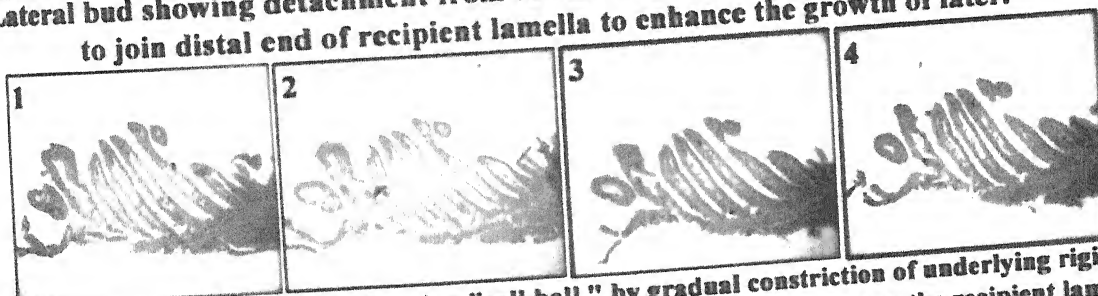
The middle lamellae contain elongated body with distal end lined by indifferent epithelium which is richly supplied with large flask shaped mucous secretory goblet cells. The submucosa is well built in the proximal and middle part but detached from the basement membrane in an irregular manner in the distal region of these lamellae (Plates-10, 11).



A. Stages depicting emergence of minor lamella from mother lamella by the process of outpushing of submucosa and mucosa of mother lamella.



B. Lateral bud showing detachment from the mother lamella and gradually elongating to join distal end of recipient lamella to enhance the growth of later.



C. The distal end of lamella discharging "cell ball" by gradual constriction of underlying region. It later joins to the subsequent lamella and contributes lamellar contents to the recipient lamella.

Plate-7 : Section passing through old and worn out set of posterior lamellae of *B. bagarius* showing abnormal elongation, widening, curving, fragmenting, crypting, swellings and other mucosal surface specification. Magnification 100 X.

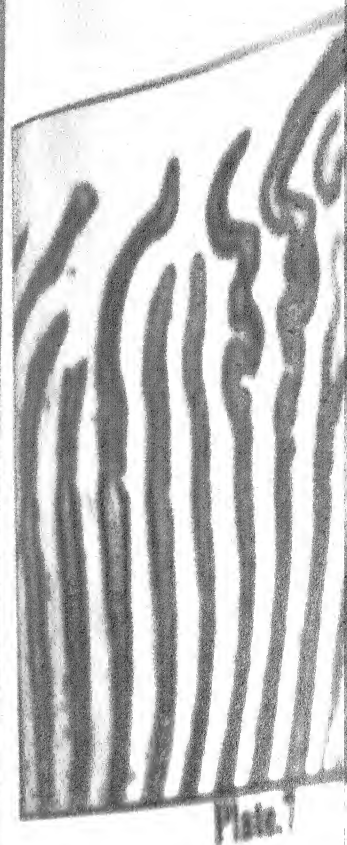


Plate-8 : Magnified section of *B. bagarius* passing through the curving, crypting, broadening, capillary accumulation, goblet cell activity and formation of different deepenings in the form of crypts accommodating primary neurons send their dendritic end to the respective lumens. Magnification 450 X.



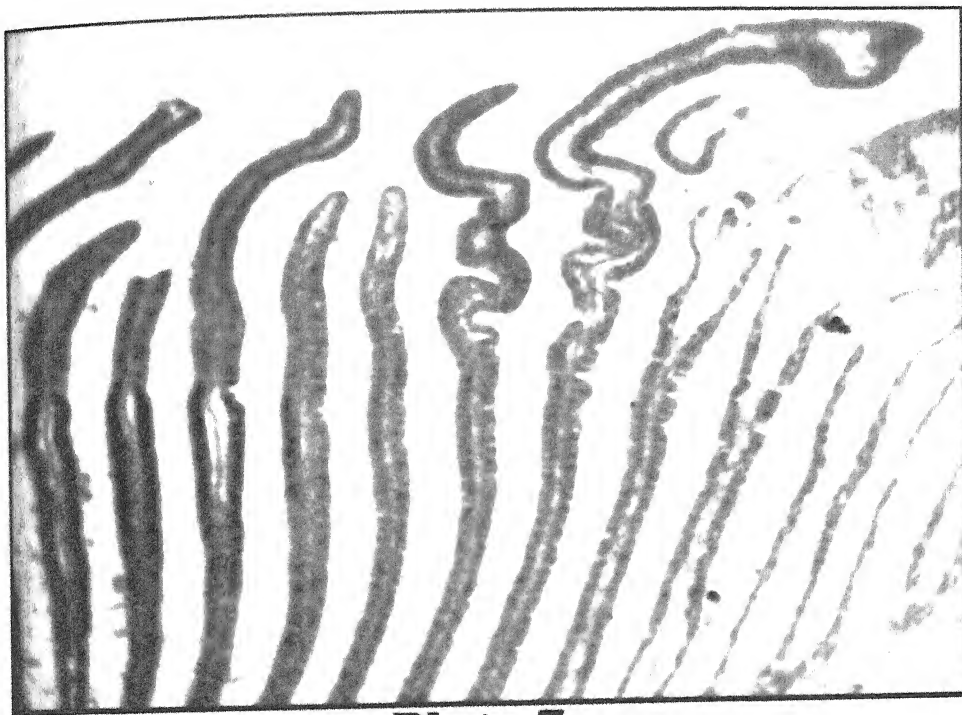


Plate.7



Plate.8

The hinder ones are old and worn out set of lamellae with enormously enlarged submucosa which has fragments of blood capillaries and loose collagen connective tissue (Plate-7). They are broad and short, lined with nonciliated cuboidal supporting cells (SC.) and mucous secretory goblet cells (GC.) throughout their surface. The receptor cells are distributed upto the middle of each hinder lamellae, though they are less in number (Plate-9).

The curved (CUR. LAM., Plate-12) and minor lamella (MIN. LAM., Plates-13,14,19A) can be observed in the middle and initial lamellae respectively. The formation of minor lamella takes place in the proximal end of the lamella, forming its minor offshoot which remains attached with it. The curving is noticed in the distal end of the initial lamella, where the whole of distal tip becomes curved in the form of 'U' shaped structure.

The distal tips of the middle and hinder lamellae undergoes the process of discharging their lamellar contents in the form of "Cell balls" (C. BALL) which contains all the contents of the olfactory epithelium (Plates-15,19B). They get discharged from the distal tips by gradual constriction (CONS., Plate-15,19B) of the underlying region of the lamella. The "Cell Balls" are arranged against the distal end of the lamellae in a regular manner, showing their gradual disintegration. Thus, this may be probably assumed that they might be supplying their cellular contents as nutrients to the other part of the olfactory rosette (Plate-19C).

The bud formation is observed in the hinder lamellae, which originate from the lateral surface of the distal end. This bud contains living contents of the olfactory epithelium and shows gradual

Plate-9 : Magnified section passing through the middle region of hinder lamellae of *B. bagarius* showing the presence of goblet cells, swelling in submucosa with rich concentration of blood capillary, connective tissue fibre and other cellular elements of submucosa. Primary neurons are richly supplied and spindle shaped receptors are also visible. Magnification 750 X.

CONN. TIS.FIB.	-	Connective tissue fibres
FOL.OLF.	-	Folium olfactorium
GC.	-	Goblet cell
MSA.	-	Mucosa
PN.	-	Primary neuron
SMSA.	-	Sub mucosa
SR.	-	Spindle shaped receptor

Plate-10 : Magnified section through distal and proximal end of the lamellae of *B. bagarius* showing attachment with the peripheral wall of the olfactory chamber with clear cut submucosa differentiation in between proximal and distal zones of lamella. Magnification 750 X.

BC.Z.	-	Basal zone
DIS.E. LAM.	-	Distal end of lamella
GC.	-	Goblet cell
PRO.E.LAM.	-	Proximal end of lamella
SC.	-	Supporting cell
SR.	-	Spindle shaped receptor

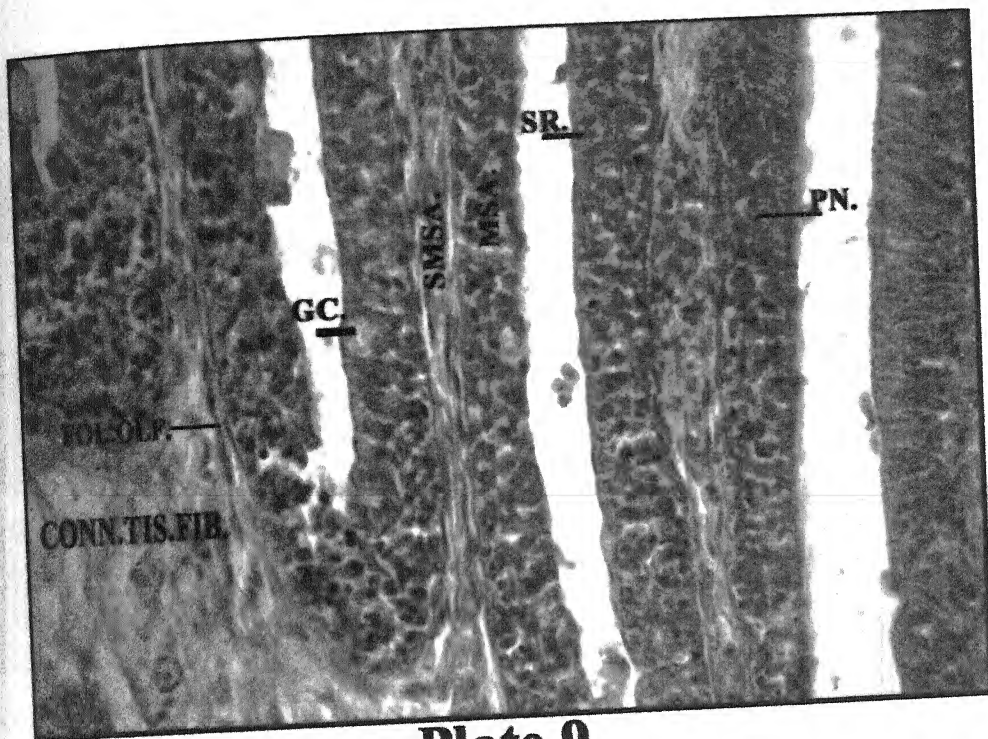


Plate.9

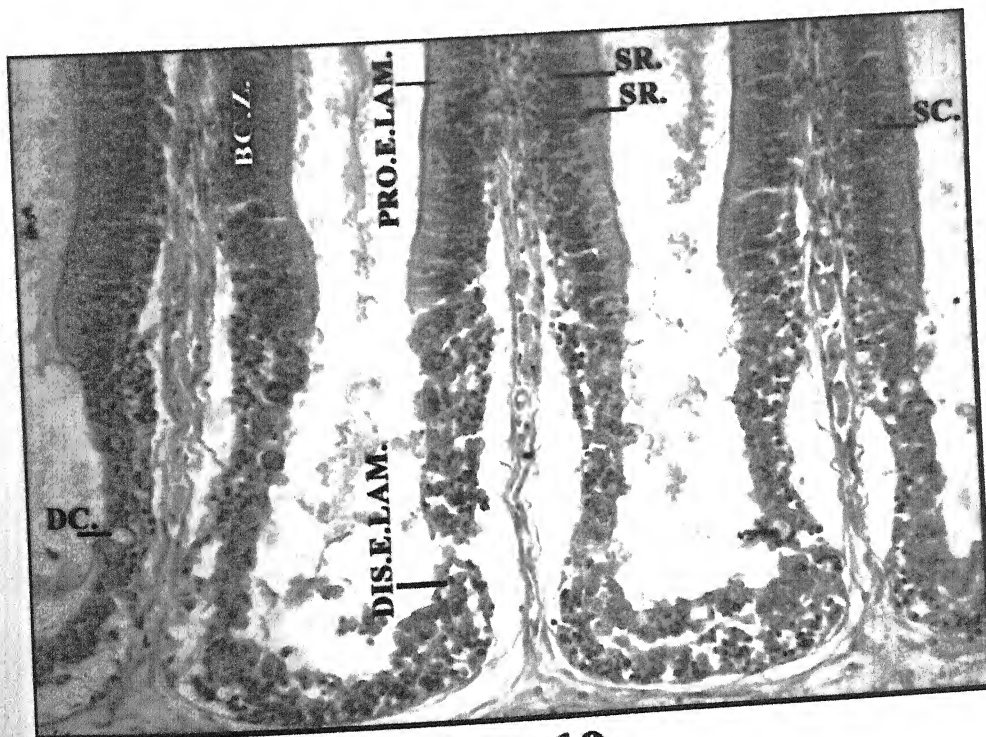


Plate.10

Plate-11 : Magnified section passing exclusively through distal zone of lamella of *B. bagarius* showing broad, loosely arranged submucosa, fragment of connective tissue fibres, blood capillaries and other submucosa elements. Goblet cells with prominent theca depicting mucous discharging activity. Primary neurons and spindle shaped receptors are also visible. Magnification 750 X.

BCP.	-	Blood capillaries
CONN. TIS.FIB.	-	Connective tissue fibres
GC.	-	Goblet cell
MU.	-	Mucous
PN.	-	Primary neuron
SMSA.	-	Sub mucosa

Plate-12 : Transverse section of the one half of the rosette of *B. bagarius* passing through the region of initial lamellae. The curved distal end of the lamella and swellings of submucosa are also visible. Magnification 100 X.

CONN. TIS.FIB.	-	Connective tissue fibres
CUR. LAM.	-	Curved lamella
DIS.E. LAM.	-	Distal end of lamella
INT.LAM.SP.	-	Inter lamellar space
MSA.	-	Mucosa
NMN.FIB.	-	Non medullated nerve fibre
PRO.E.LAM.	-	Proximal end of lamella
RPH.	-	Raphe

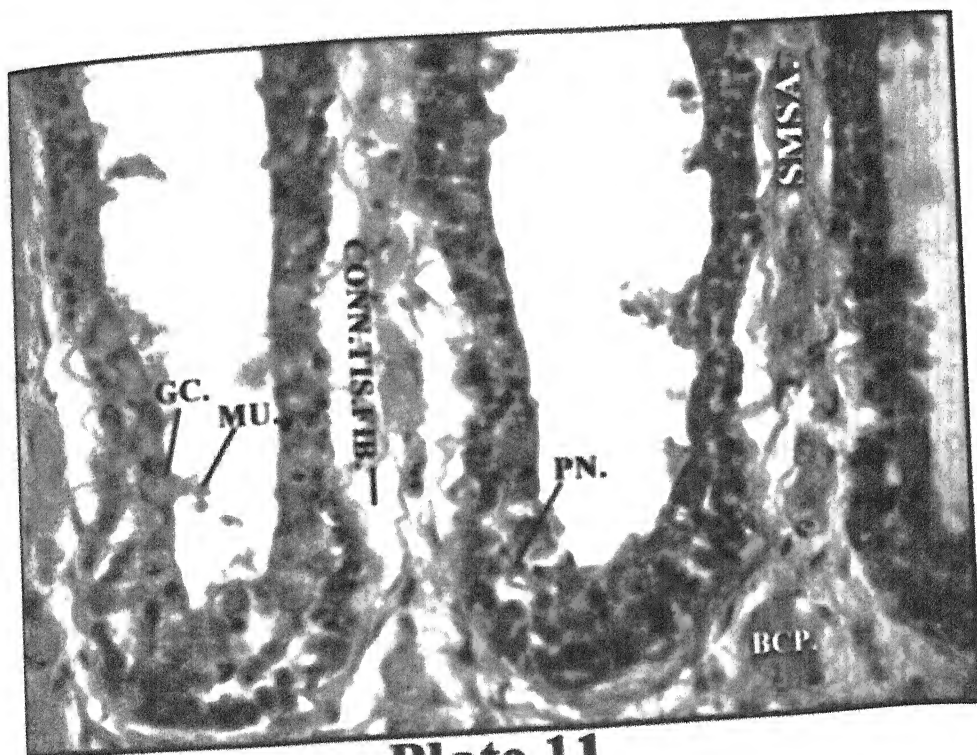


Plate.11

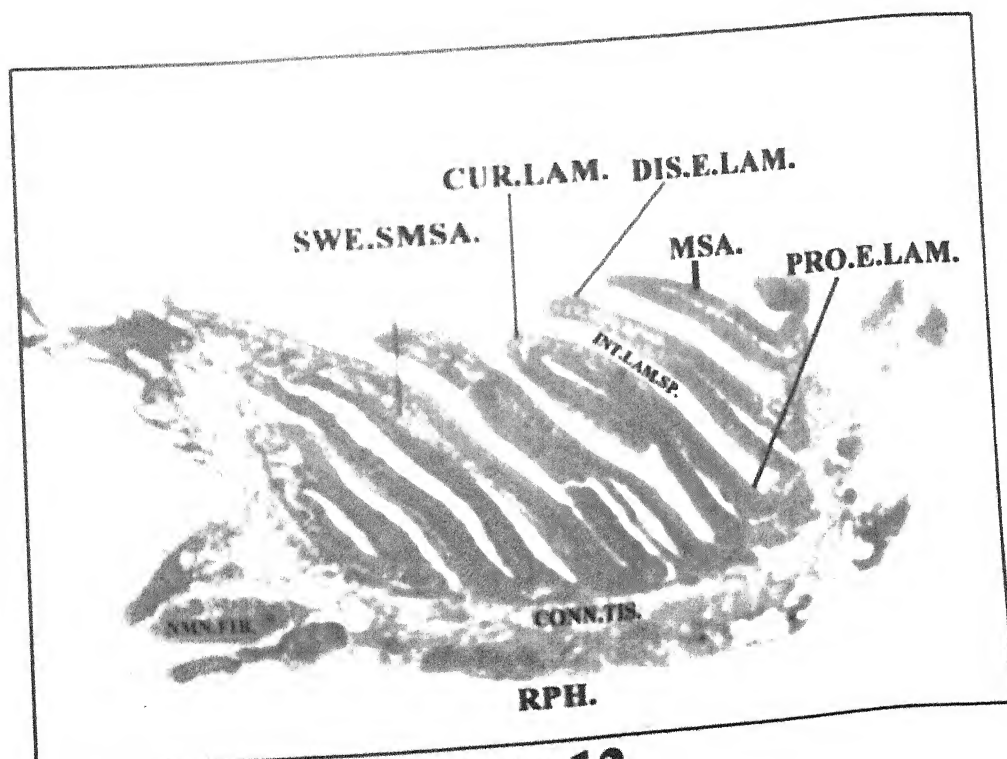


Plate.12

attachment on the adjacent lamella after being detached from the mother lamella (MOT. LAM.). In this process the recipient lamella (REC. LAM.) and the bud elongate (ELO.BUD) gradually to join each other and ultimately the later becomes fixed on the former. This cause immediate enlargement of recipient lamella with the result of the addition of the content of olfactory epithelium in the form of bud (Plates-16,19B)

The submucosa swells abnormally in the initial and recipient lamellae, which are in the process of curving and attachment with the bud respectively (Plates-12, 16). This may be due to the accumulation of basal cells, connective tissue, blood capillaries and other epithelial contents required for elongation of lamella for attachment with the bud or curving.

On the basis of distribution of supporting and sensory cells, the lamella of *B. bagarius* can be divided in following zones :

Proximal zone : It extends on either sides of raphe upto the middle region of the olfactory rosette. The anterior and middle lamellae of this region have columnar ciliated epithelium with rich supply of receptor cells. This region is devoid of mucous secretory goblet cells.

Distal Zone : The distal zone of the lamella is composed of non-ciliated columnar supporting cells (S.C.). This zone is nonciliated but mucous secretory goblet cells (G.C.) are richly present. The central core of this region is supplied with pigment cells.

The following cell types may be identified in the olfactory epithelium of *B. bagarius* : Supporting or sustentacular cells, receptor cells; goblet cells and basal cells. The cellular components and their nuclei are arranged in the series from inner (or basal) to outer (or

peripheral) margins in the following manner. The inner, most position next to basement membrane (BM.) is occupied by the basal cells (BC.) having rounded or irregular nucleus. These are followed by the nuclei of spindle shaped receptor cells (SR.) and then nuclei of supporting cells. Peripheral or outer zone is filled with the distal end of the supporting cells and dendrites of the receptor cells. The goblet cells are confined in the hinder lamellae or in the distal end of all lamellae intermingled with supporting cells.

Supporting cells :

They are columnar and cuboidal, arranged perpendicular to the central core of the lamella and contributes in the formation of greater bulk of the olfactory epithelium. These cells can be distinguished in following types : ciliated supporting cells; nonciliated supporting cells and transitionary supporting cells.

Ciliated supporting cells (Cl. SC.) are tall and richly ciliated. They are confined in the proximal and middle region of the initial and middle lamellae. The arrangement of these cells in the olfactory epithelium is very compact and no intercellular spaces can be seen among them. The columnar cells are made of proximal or inner limb and distal or outer limb. The later is broad and elongated, extending upto the peripheral surface of the lamella while the former is short, inconspicuous and extends upto the basement membrane. The cytoplasm of these cells frequently show granulated appearance and granules tend to become concentrated at the distal tip. The distal end of ciliated supporting cells bear cilia (OCI) which project into the interlamellar spaces. The spherical or oval nucleus of the ciliated supporting cell (NU.SC.) lies in the proximal part of inner limb. A

centrally situated nucleolus is clearly visible and chromatin material is evenly distributed in karyoplasm. The nucleus of ciliated supporting cells takes sharp stain of haematoxylin.

Nonciliated supporting cells (NCI. SC.) are confined in the distal regions of the initial and middle lamellae but the epithelium of hinder ones is mainly made up of these cells. They are short columnar and nonciliated provided with oval nucleus. The distal or outer limb is short, broad and terminates in the peripheral surface of the lamella by an expanded tip. The proximal or inner limb is inconspicuous but distal or outer end is prominent and broad. The nucleus lies somewhere in the proximal or inner side of the cell. The nuclei of these cells lie at different levels of the epithelium with clear nucleus and a uniform distribution of chromatin material.

The olfactory epithelium of hinder lamellae is mainly constituted of nonciliated cuboidal supporting cells. They are made up of short and broad distal limb and bears darkly staining rounded nucleus. The cuboidal supporting cells are compactly arranged along the peripheral surface of the mucosa which provide insulation to the dendrite of spindle shaped receptor cells (DN.SR.) The centrally placed nucleolus and chromatin material are clearly visible in the nucleus of cuboidal supporting cells.

Some of the nonciliated supporting cells are positively muciferous and are denominated as transitional supporting cells (T. SC.) The distal or outer limb of these cells become ovoid pushing the cytoplasmic and nuclear content towards the proximal or inner side. The cytoplasmic and nuclear contents remain compressed while the distal part gradually filled with the mucin forming contents.

Receptor cells :

The receptor cells are confined in the proximal and middle part of all the lamellae, however, they are highly concentrated in the middle regions. The distal regions of all the lamellae show complete absence of the receptor cells. The receptor cells are interspersed among the ciliated columnar and nonciliated cuboidal supporting cells. Their grouping in the form of olfactory bud is not observed in the olfactory epithelium of *B. bagarius*. The receptor cells have slender body with scanty cytoplasm, surrounding the elongated oval nucleus. It takes good stain of haemotoxylin but slightly lighter than the nuclei of the surrounding supporting cells. Nucleus and chromatin material are clearly visible in the nuclei of receptor cells. These cells are situated deep in the olfactory epithelium and send their elongated dendrite to the peripheral surface of the lamella. The dendrites can easily be identified from the distal ends of supporting cells due to their filamentous nature. The olfactory cilia (OCI.) are seen projecting out from the distal tip of dendrite of receptor cells and they are longer than the cilia of supporting cells. It is difficult to trace the axonal end of receptor cells but careful staining and sectioning of material reveal few of them very clear. The axonal end of all the receptors meet along the basement membrane to form folium olfactorium (FOL.OLF.) which ultimately join nonmedullated nerve fibres (NMN.FIB.) passing through the raphe (Plates-6,9,10).

In *B. bagarius* two types of receptor cells are found, namely. Primary neurons and spindle shaped receptors. The distribution of these receptor cells varies in different regions of the lamella. The

Plate-13 : Vertical section of rosette of *B. bagarius* showing emergence of basal minor lamella from mother lamella and bifurcation of submucosa is visible, which is indicated by arrow. magnification 450 X.

INT.LAM.SP.	-	Inter lamellar space
MIN.LAM.	-	Minor lamella
MSA.	-	Mucosa
SMSA.	-	Sub mucosa
UN.CRY.	-	Unexposed crypt

Plate-14 : Vertical section of rosette of *B. bagarius* showing the growth of minor lamella and unexposed crypts formed by the fusion of mucosa of mother and minor lamella. Submucosal division is indicated by arrow. Magnification 450 X.

MIN.LAM.	-	Minor Lamella
MOT.LAM.	-	Mother lamella
SMSA.	-	Sub mucosa
UN.CRY.	-	Unexposed crypt

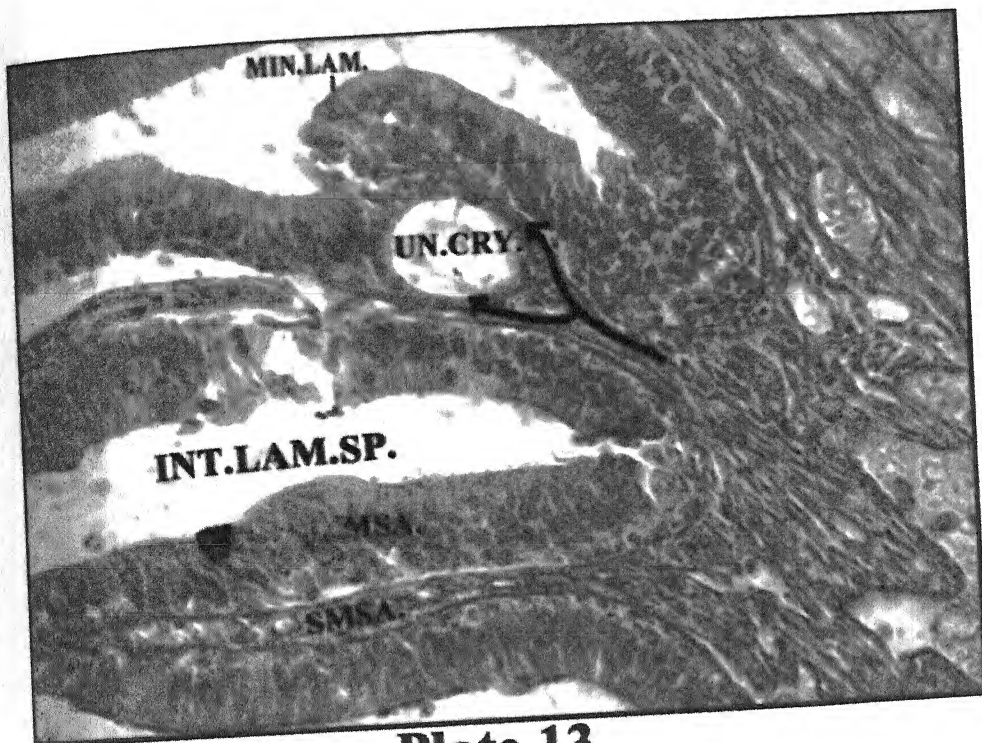


Plate.13

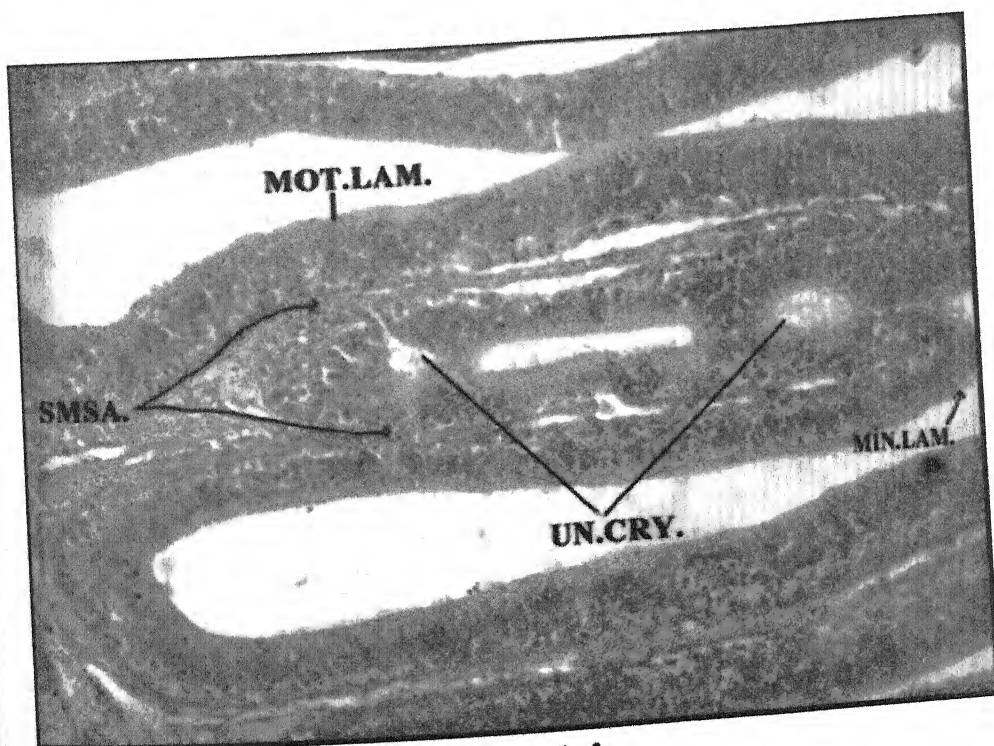


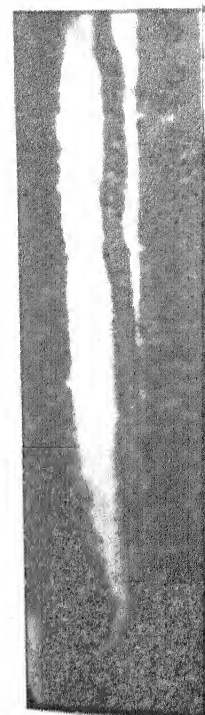
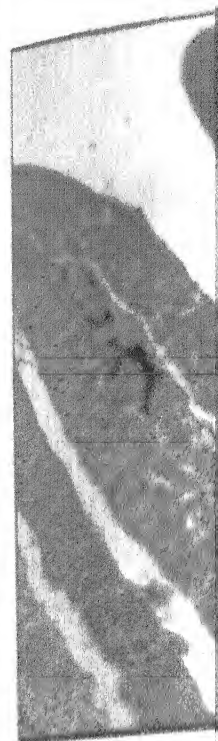
Plate.14

Plate-15 : Transverse section of one half of the rosette of *B. bagarius* passing through hinder lamella and showing a stage of discharge of cell ball by the process of gradual constriction of underlying region. Magnification 450 X.

C.BALL.	-	Cell ball
CONS.	-	Constriction
DET.C.BALL	-	Detached cell ball
INT.LAM.SP.	-	Inter lamellar space
GC.	-	Goblet cell

Plate-16 : Vertical section of *B. bagarius* showing joining of cell ball with recipient lamella after getting detached from mother lamella. Junctional morphogenetic activity is clearly visible. Magnification 450 X.

ELO.BUD.	-	Elongated bud
FU.LAM.	-	Fused lamella
INT.LAM.SP.	-	Inter lamellar space
MSA.	-	Mucosa
MU.	-	Mucous
REC.LAM.	-	Recipient lamella
SMSA.	-	Sub mucosa



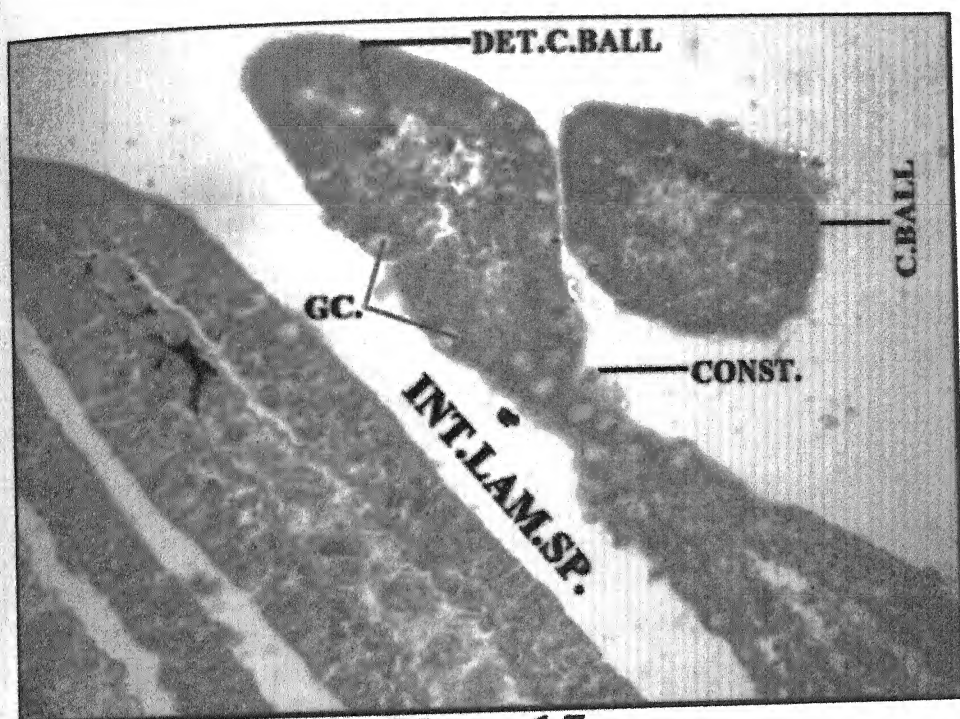


Plate.15

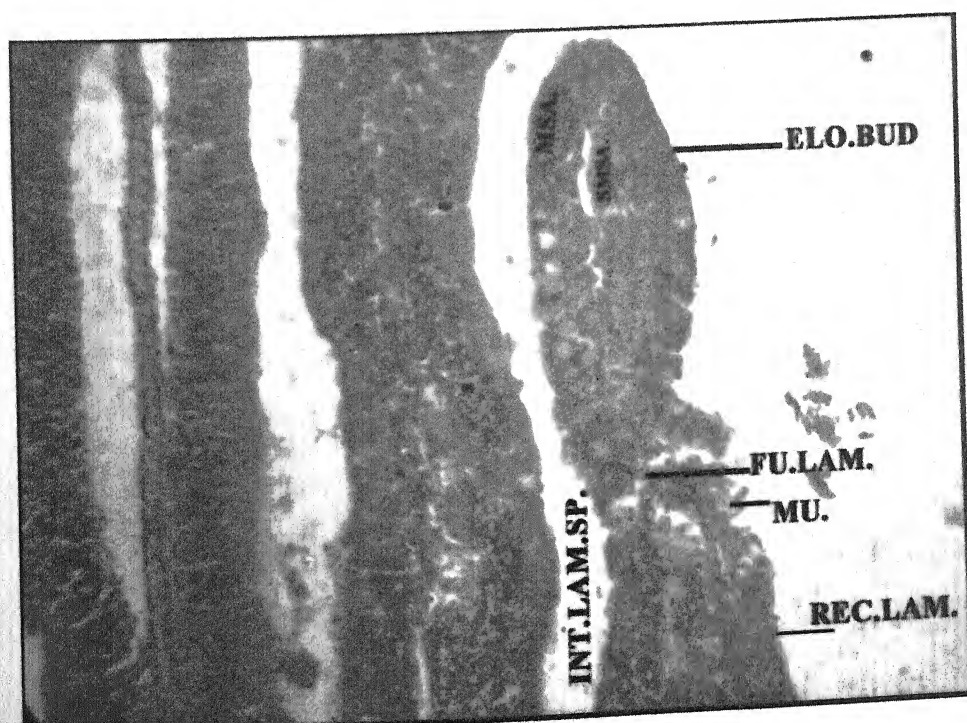


Plate.16

spindle shaped receptors and primary neurons also forms synapse, which can be easily seen in the mucosal zone. (Plates-6,9,10).

Goblet cells :

The mucous secretory goblet cells (GC.) are confined in the distal region of the initial and middle lamellae (Plates-10,11,15,16), but can be encountered any where in hinder ones (Plate-9). The proximal and middle regions of initial and middle lamellae are devoid of the mucous secretory goblet cells. A fully developed goblet cell bears an apical end, filled with pale mucigen droplets and slender basal end containing compressed nucleus and small amount of deeply stained cytoplasm. The apical part of these cells has an expanded cup called theca, which is filled with secretory droplet. It becomes empty after discharging the mucous contents in the interlamellar spaces. The proximal or inner limb is stem like extending upto the basement membrane (BM.). It is hard to observe the presence of nucleous and chromatin material in nucleus due to high degree of compression.

The goblet cells can be identified as : megagoblet cells (MG.) and microgoblet cells (MIG) in the olfactory epithelium of *B. bagarius*. The former are larger and flask shaped and are formed by the transformation of the nonciliated columnar supporting cells. The nuclear and cytoplasmic contents are pushed in the form of triangular darkly stained mass (NU. MG.) situated proximally in the cell body. They generally lie on the peripheral margin of lamella either filled with mucous or empty theca (TH. GC.) after its dischargement.

The microgoblet cells in *B. bagarius* are transformed from the cuboidal supporting cells of hinder lamellae. The are present on the peripheral or outer surface of the olfactory epithelium and generally

bears an outwardly projected beak like structure. They are having nearly oval theca and compressed nuclear body. They are frequently seen in the hinder lamellae and discharged part of the lamellar contents (cell ball and bud, C. Ball and Bud, Plates-15,16,19B,C).

Basal cells :

They are rounded with irregular branching processes. Each cell has rounded, irregular and darkly staining nucleus with faintly visible and chromatin material. The cytoplasm form a thin border around the nucleus. The basal cells (BC.) are sparse and scanty in the proximal and middle regions of the initial and middle lamellae and are arranged in a single row (Plates-6,10). In the distal region of all lamellae and in hinder ones, these cells are irregularly arranged forming three to four rows of basal cells just above the basement membrane (Plate-11). Their rich aggregation can be observed in cell ball (C. Ball, Plate-15) and bud (BUD, Plate-16) forming lamellae. The basal cells show their specific migratory tendency towards the formations of the cell ball and bud. At the places of above formations, they are seen line up and take position in the preparations for their eventual transformation and migration.

Central core or submucosa :

The central core or submucosa (SMSA). is lined on either sides by a well defined basement membrane (BM). It is filled with collagen of connective tissue (CONN. TISS. FIB.) and long areolae (ARE., (Plate-4) are present in between the facia of collagen connective tissue (Plates-4,6,10). In the distal region of the lamellae the aerolar connective tissue is converted into dense connective tissue in which no areolae are observed (Plate-10). The submucosa of the hinder lamellae

becomes enormously enlarged causing damage to the connective tissue fibre and blood capillaries (BCP, Plates-4, 9). The fibroblast cells (FIB., Plate-9) are commonly observed in the central core of the distal regions of the initial and middle lamellae and in the hinder lamellae their rich supply is noticed. The histocytes (HIS.) and basal cells (BC.) can be observed in the connective tissue. Branched pigment cell (PIG. C. Plates-8,17,18) are seen in the submucosa of middle and hinder lamella, which are confined in the middle and distal regions of these lamella. The blood capillaries (BCP., Plates-4,8,9,17,18) transverse through the central core and at certain places their swellings (SWE., Plate-8,9,10) can be observed. The nonmedullated nerve fibres (NMN. FIB., Plate-4) extend through the central core along the basement membrane. The central core of all the lamellae is in continuation the central core the raphe and all the vascular, nervous and cellular supply is passed to the lamellae through it (Plates-4,5,9,10,11).

The raphe:

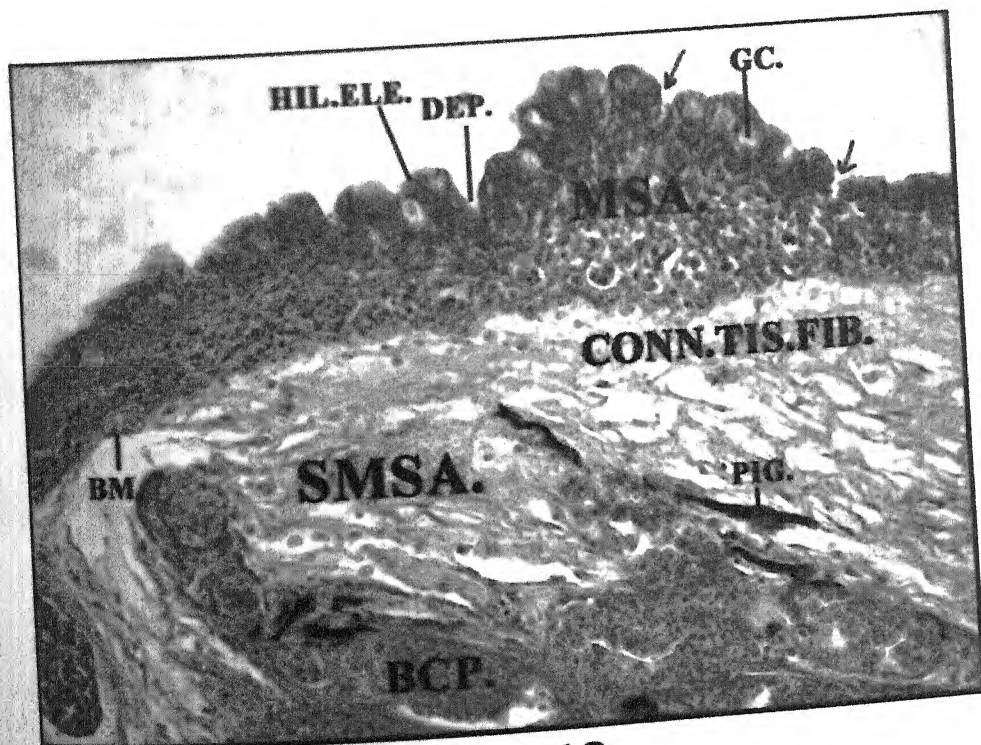
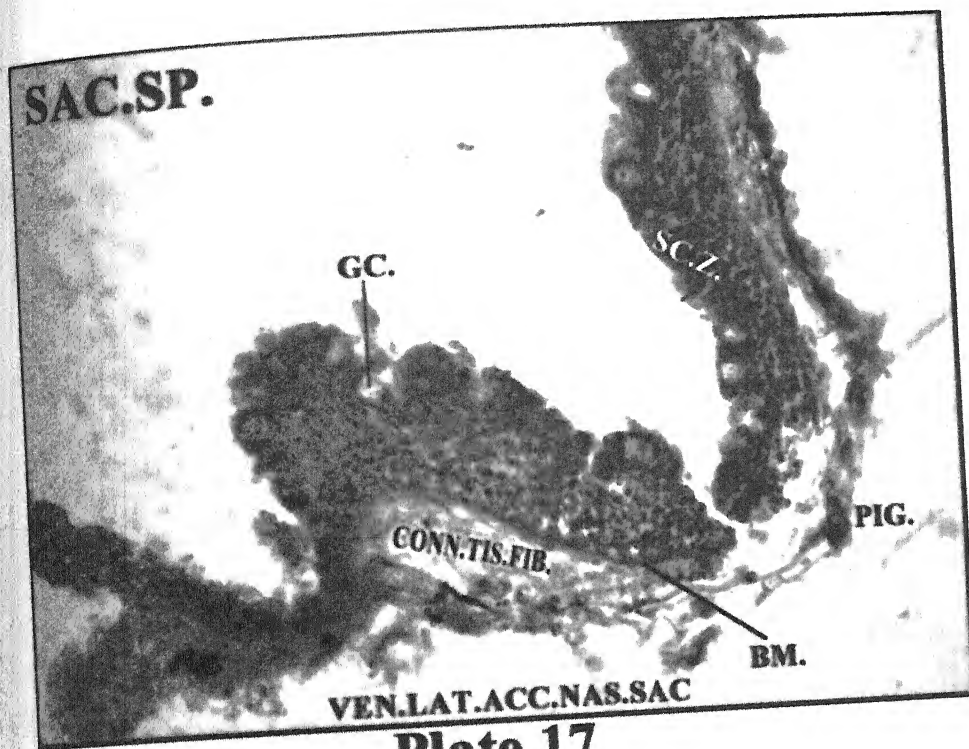
The raphe (RPH.) is made up of simple columnar epithelium which lies on either sides of the well demarcated basement membrane (Plates-4,5,12,13) cells bear darkly stained nucleus (NU. SC.), situated just above the basement membrane in a uniform level. The elongated peripheral surface of the olfactory epithelium of raphe and cytoplasm of columnar cells is homogeneous. No other cellular component is seen in the olfactory epithelium of raphe of *B. bagarius*. The central core of submucosa of the raphe is spacious and is filled with connective tissue (CONN. TIS.). The nonmedullaated nerve fibres (NMN. FIB., Plate-4.) are observed below the basement membrane

Plate-17 : Section passing through the ventrolateral accessory nasal sac of *B. bagarius* showing thick basal zone, wavy supporting zone, hillock elevation and depression with prominent goblet cell activity. Elastic connective tissue fibre are also seen traversing through the submucosa of sac. Magnification 450 X.

BM.	- Basal membrane
CONN. TIS.FIB.	- Connective tissue fibres
GC.	- Goblet cell
PIG.	- Pigment cell
SAC.SP.	- Sac Space
SC.Z.	- Supporting zone
VEN.LAT.ACC.NAS.SAC	- Vento lateral accessory nasal sac

Plate-18 : Magnified section of olfactory epithelium of ventrolateral accessory nasal sac of *B. bagarius* showing abnormally broad, thick submucosa with dense elastic connective tissue supply, pigment cell, blood capillary system, and all other prominent cellular elements of submucosa. Supporting zone is more pronounced showing prominent hillock elevation and depressions with prominent goblet cell activity. Magnification 750 X.

BCP.	- Blood capillaries
BM.	- Basal membrane
CONN. TIS.FIB.	- Connective tissue fibres
DEP.	- Depression
GC.	- Goblet cell
HIL.LE.	- Hillock elevation
MSA.	- Mucosa
PIG.	- Pigment cell
SMSA.	- Sub mucosa



which send their nervous supply to lamella. The blood capillaries and their direction of supply can be seen in the raphe of *B. bagarius*. The fibroblasts (FIB.), histocytes (HIS.) and basal cells (BC.) are rarely seen in the connective tissue of raphe..

Accessory nasal sac:

The ventrolateral accessory sac of *B. bagarius* is made up of non ciliated cuboidal epithelium. The epithelial lining of the sac is wavy and shows hillock elevations (HIL.ELE.) and depression (DIP., Plates-17,18). It consists of cuboidal supporting (SC.) cells, rounded goblet cells (GC.) and basal cells (BC.).

The cuboidal cells are situated in the periphery with darkly stained oval nucleus. They can be seen in two or three rows in elevated regions of the epithelium. The goblet cells are rounded, neckless and found embedded in the peripheral epithelial surface. They can also be observed with empty theca after discharging their mucous contents. They can also be seen in two or three rows in regions of elevations. The basal cells lie in three or four rows just above the basement membrane. In the elevations, basal cells are accumulate in large number and show their migratory tendency towards the periphery.

The wavy basement membrane lies just below the basal cells and is followed by elastic connective tissue. The elastic fibres are loosely cemented with matrix and are also followed by thin collagen fibres. The fibroblasts and basal cells can also be observed within the elastic and collagen connective tissue fibres. Blood capillaries and nonmedullated nerve fibres are present in the connective tissue of the accessory sac of *B. bagarius* (Plate-17,18).

The number of sac layers vary with the distension of accessory sac. In a normal condition, The cuboidal epithelial and basal cells are accumulated in 9 - 11 layers (Palte-17). The elastin fibres and basement membrane is wavy, however, in a distended condition the accessory sac consists of 2-3 layers of basal cells. The basement membrane and elastic fibers are stretched in distended condition (Plate-18).

Ecological co-efficient :

The usual methods are employed to calculate the ecological co-efficient in fishes varying from 140mm to 270mm in total length. The length of brain and number of lamellae undergo considerable increase with respect to the size of the fish (Table-7). The size of mesencephalon ranges from 1.98mm to 2.44mm in length where as the telencephalon varies from 2.13mm to 2.96mm (Table-7).

The areas of both retinae and those of rosette of both the sides are calculated by Teichmann (1954) method and is further modified by Rahmani and Khan (1981). It is found that former ranges from 14.12mm² to 39.24mm² and that of later from 167.54mm² to 485.90mm² (Table-7). The area of both the rosettes is higher where as the retinal area is insignificant showing thereby feebly developed optic faculty. The olfactory centre in the brain also adds further weightage to the efficiency of the olfactory faculty. *B. bagarius* is, therefore, be placed under "nose-fish" category, where the olfactory faculty plays its significant role in the habit of the fish, such as location of food and fright reactions etc. *B. bagarius* is a nocturnal fish and lives in dark places which supports the findings that the fish under observations

needs a better developed olfactory faculty rather than retinal (optic faculty).

The route of water circulation through the olfactory chamber of *B. bagarius* :

The movement of nasal barble (NAS. BAR.) and pumping activity of the ventro-lateral accessory nasal sac, synchronously with the unidirectional beating of cilia conduct the water current through the anterior tubular nasal opening over the anterior most part of the olfactory rosette. From there the water current is directed to the central and peripheral channels of the olfactory chamber. The channels are converged posteriorly (Fig.-1B) in a narrow lamellae-less region of the olfactory rosette which is communicated by an aperture to the accessory sac, resulting the water current to the sac after crossing the entire distance of the rosette. In this course of circulation, water travels through the interlamellar spaces and each lamella is properly bathed. The compression of accessory sac causes the exit of water current from the posterior nasal opening. Valvular arrangement of posterior nasal opening can only allow the exit of water current, demonstrating unidirectional flow of water through the olfactory chamber.

The continuous and gradual flow of water through the olfactory chamber from anterior to posterior nasal opening is a regular feature in *B. bagarius*, but during forward movement it becomes more rapid. The slow passage of water current through the olfactory chamber maintains a regularity with opercular movements. This indicates that respiratory movements also help to transport water through the olfactory chamber.

Plate-1 : Lateral view of head of *T. mossambica*

ANT. NAS. OP. - Anterior nasal opening

POST. NSA. OP. - Posterior nasal opening

Plate-2 : Dissection of the head of *T. mossambica* from lateral side to show rosette insitu .

ETH.ACC. NAS.SC. - Ethmoidal accessory nasal sac

ROS. - Rosette

LAC.ACC.NAS.SAC. - Lacrymal accessory nasal sac

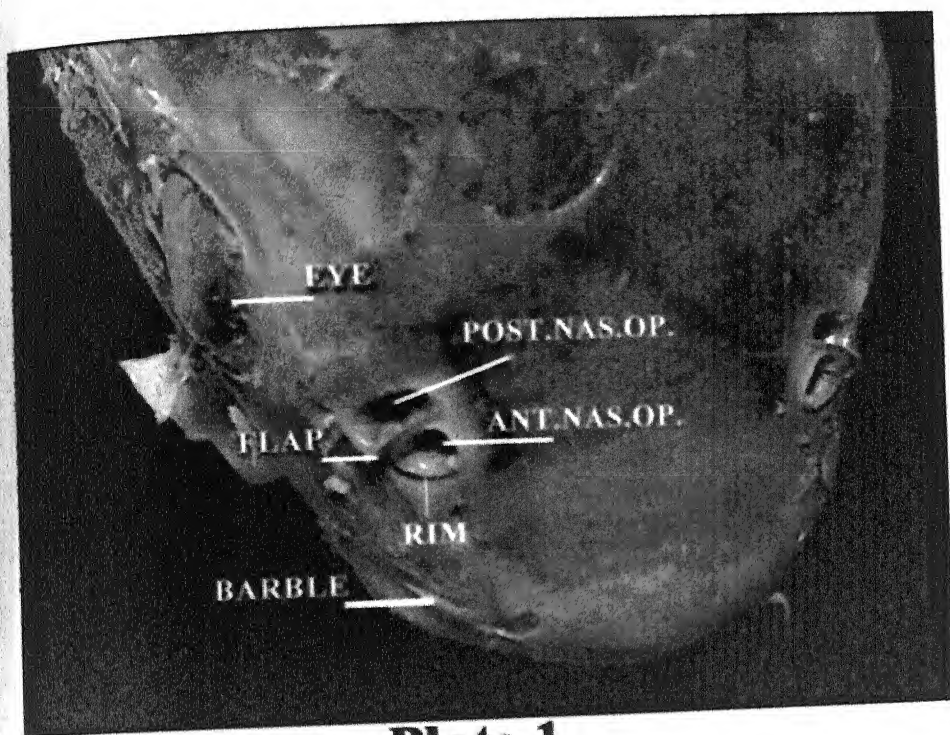


Plate.1

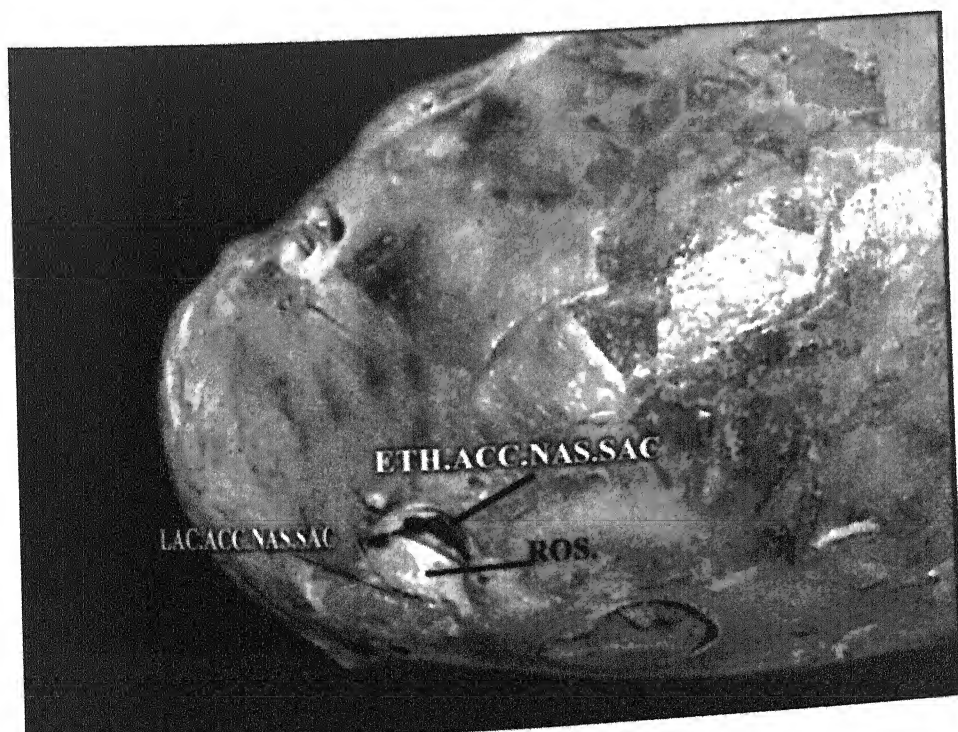


Plate.2

Histological Observations of *Tilapia mossambica*

The Olfactory organ in *T. mossambica* are comprised of a pair of olfactory chambers (OLF. CHAM.) lying dorsally on the snout, anterior to and about at the level of the eyes (Plate-1, Fig.-1A). Each olfactory chamber bears a small circular anterior and an oval posterior nasal opening (ANT.NAS.OP., POS.NAS.OP, Fig.-1A,B,C, Plate-1). The later is wide and more prominent. The olfactory chamber is somewhat quadrangular in shape, floored with the rosette (ROS.), which is having less pronounced olfactory lamellae (LAM. Fig-1B,C). The posterior nasal opening is placed higher as compared to the anterior. The later is rimmed (RIM) and non tubular, whereas, the former is having a loose fold of integument, converging half of the opening and acts as a valve. The separate openings of ethmoidal and lacrymal accessory nasal sacs (ETH.ACC.NAS.SAC., LAC.ACC.NAS.SAC.) (Fig.-1C, Plate-2) are present just below the posterior nasal opening, which allow the water circulation in both the sacs through olfactory chamber.

The area surrounding the olfactory chamber is provided with numerous chromatophores. It is occupied by a quadrangular olfactory rosette which can easily be visualized after removing the surrounding integument (Plate-2). The olfactory rosette is devoid of raphe (RPH.) and provided with fewer lamellae ranging from 7-10. The arrangement of lamellae presents a rough appearance of lotus petals, emerging out from one point and expanding at the other. The lamellae in *Tilapia mossambica* are of different type i.e. they do not have their separate formation but they are in the form of thickening, attached with the

- Fig. 1A Diagram of the lateral view of the head of *T. mossambica*
- Fig. 1B Diagram of the olfactory chamber to show the position of anterior and posterior nasal opening in *T. mossambica*.
- Fig. 1C Diagrammatic sketch of the olfactory chamber of *T. mossambica* to show the position of ethmoidal and lacrymal accessory nasal sacs. Arrows indicating the entry and exist of water through nasal openings and its course of circulation within the olfactory chamber.
- Fig. 1D A set of 1 - 10 lamellae from a rosette of *T. mossambica*

ANT. NAS. OP.	: Anterior nasal opening
ETH. ACC. NAS. SAC	: Ethmoidal accessory nasal sac.
EY.	: Eye
INTEG.	: Integument
LAC. ACC. NAS. SAC	: Lacrymal accessory nasal sac.
LAM.	: Lamellae
LAM. LESS AREA	: Lamellaeless area
OP. ETH. ACC. NAS. SAC	: Opening of ethmoidal accessory nasal sac.
OP. LAC. ACC. NAS. SAC	: Opening of lacrymal accessory nasal sac.
POS. NAS. OP.	: Posterior nasal opening.
RIM	: RIM

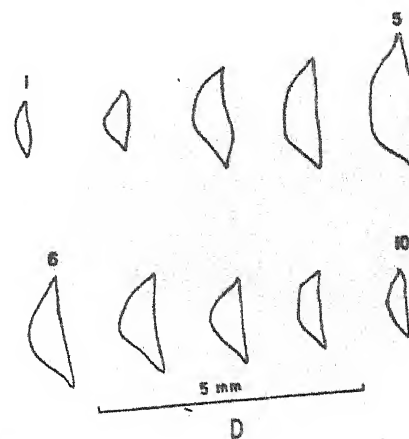
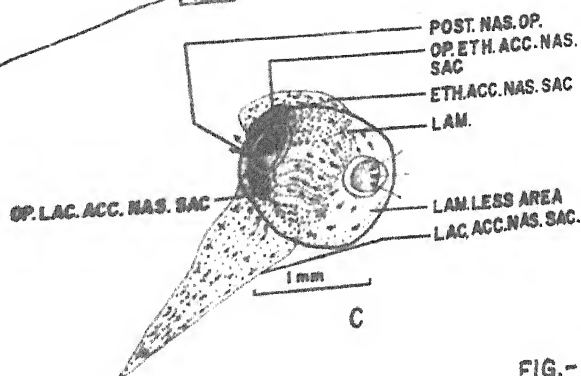
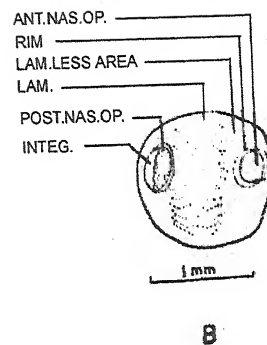
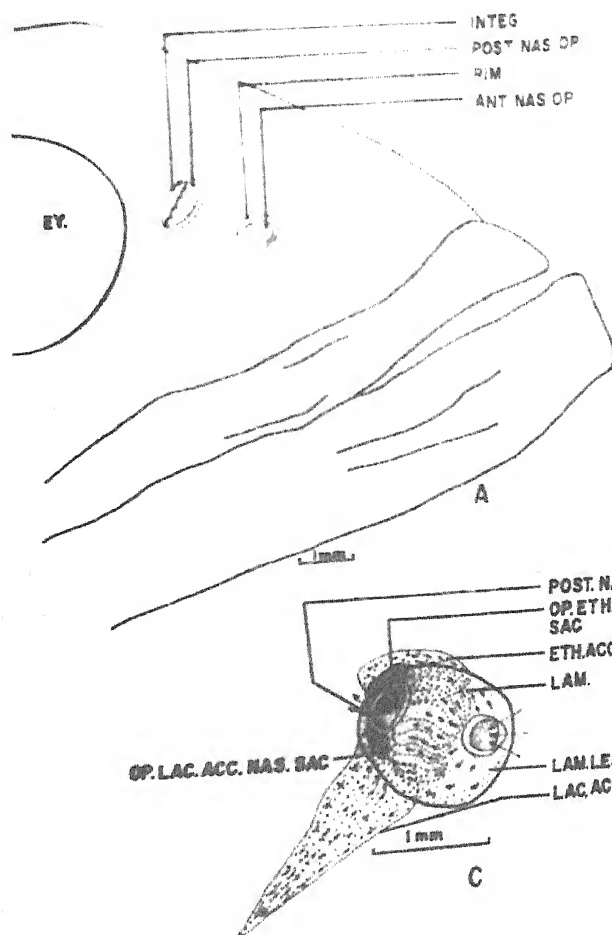


FIG.-3

Fig. - 1

Fig.2

Diagram of the dissection of head of *T. mossambica* from dorsal side to show the relationship of brain with the rosette.

CE.	:	Cerebellum
OLF. BL	:	Olfactory Bulb
OLF. LO	:	Olfactory lobe
OLF. TR	:	Olfactory Tract
OP. L	:	Optic lobe
ROS.	:	Rosette

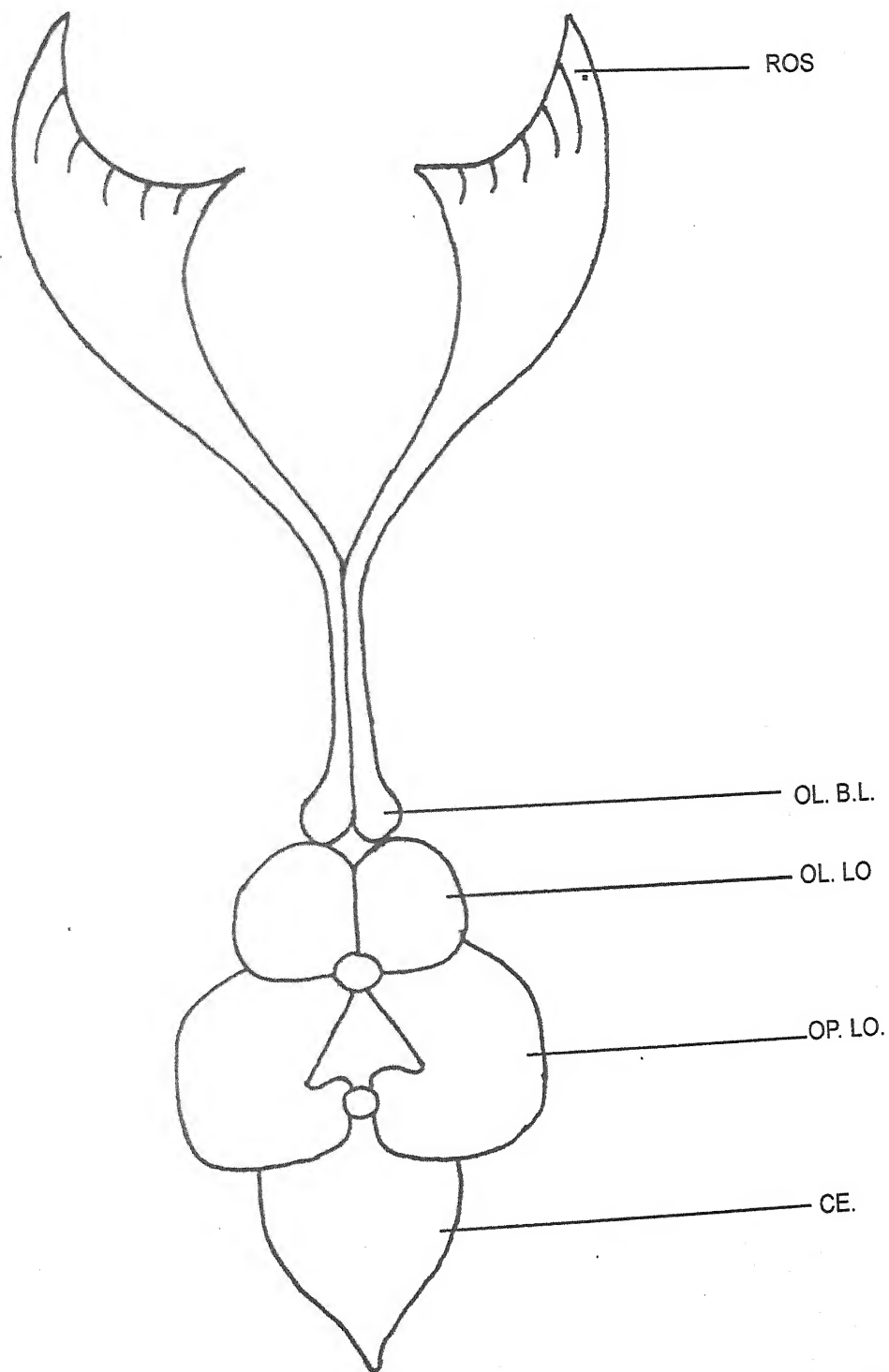


Fig. - 2

floor of olfactory chamber. The number of lamellae shows an increasing trend with the growth of the fish. The linguiform processes are wanting in *i*. The lamellae are arranged almost parallel to the body axis (Fig.-1B, C, Plate-2).

T. mossambica possesses a pair of well developed accessory nasal sac, associated with each olfactory chamber. The sacs are situated in relation to ethmoid and lacrymal bones, consequently, they are named as ethmoidal and lacrymal accessory nasal sac respectively. The opening of the former is visible in the intact fish through the posterior nasal opening (Plate-2). It is oval in shape. The wall of the sac is extremely thin and flexible. The opening of lacrymal sac is partly visible through the posterior nasal opening. It is smaller as compared to the opening of ethmoidal sac.

When the mouth is tightly closed, the opening of ethmoidal sac becomes slit like and it remains in its maximum expansion. When it is opened but the premaxilla is not extended, this opening remains slit like. However, when the premaxilla is protruded out and its extremely long ascending processes is extended rostrally, the opening of the ethmoidal sac becomes stretched and its volume is effected with the elevation of ethmoid. During this protrusion, the lacrymal sac is stretched extremely but narrows in its size causing expulsion of water into the olfactory chamber from both the sacs.

Careful removal of the long ascending process of the premaxilla, nasal, frontal and the muscles from the dorsal side, expose the olfactory nerve (OLF.N.) and the brain. The olfactory bulbs (OLF.B.) are small and attached to the forebrain. Therefore, they are sessile type. The olfactory lobes (OLF.L.) are larger and closely attached with

Plate-3 : Transverse section passing through the olfactory chamber of *T. mossambica* depicting lamellar arrangement, interrelation with ethmoidal and lacrymal accessory nasal sacs along with the visibility of crypts, cuneiform, filiform, fungiform and other elevations. Magnification 50 X.

DIS.E. LAM.	-	Distal end of lamella
ETH.ACC. NAS.SC.	-	Ethmoidal accessory nasal sac
LAC.ACC.NAS.SAC.	-	Lacrymal accessory nasal sac
MSA.	-	Mucosa
PRO.E.LAM.	-	Proximal end of lamella
SMSA.	-	Sub mucosa

Plate-4 : Transverse section passing through olfactory rosette of *T. mossambica* showing lamellar arrangement and other microformations. Magnification 100 X

ARE.	-	Areolae
BCP.	-	Blood capillaries
BM.	-	Basal membrane
BC.Z.	-	Basal zone
CONN. TIS.FIB.	-	Connective tissue fibres
CRY.	-	Crypts
CUN.	-	Cuneiform
DEP.	-	Depression
ELE.	-	Elevation
FIL.	-	Filiform
FUN.	-	Fungiform
HIN.LAM	-	Hinder lamella
INI.LAM.	-	Initial lamella
MID.LAM.	-	Middle lamella
MSA.	-	Mucosa
SC.Z.	-	Supporting zone
SMSA.	-	Sub mucosa
W.OLF.CHAM.	-	Wall of olfactory chamber

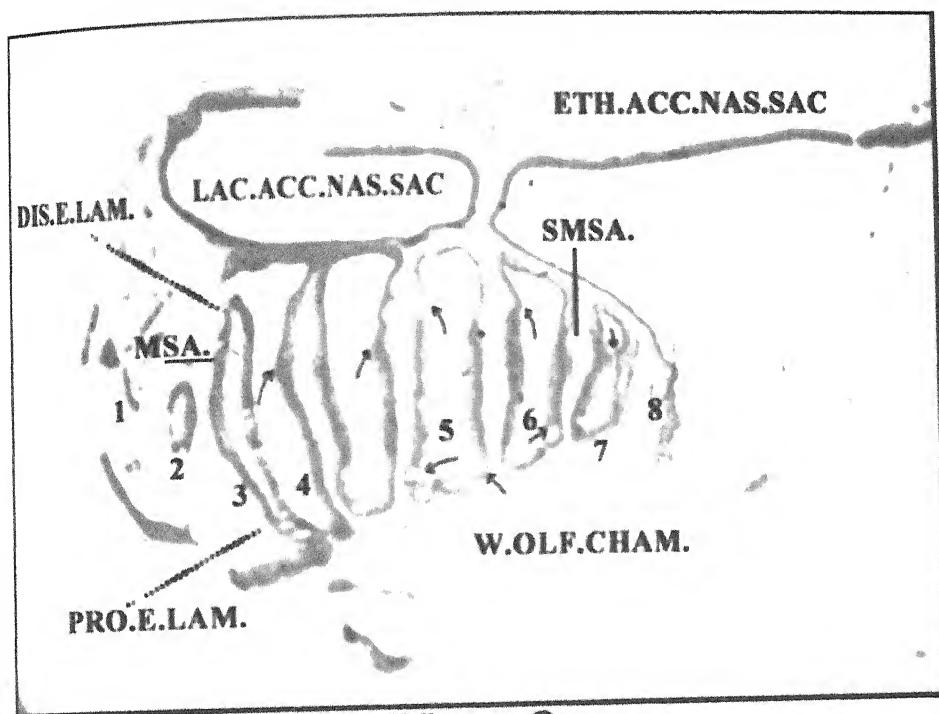


Plate.3

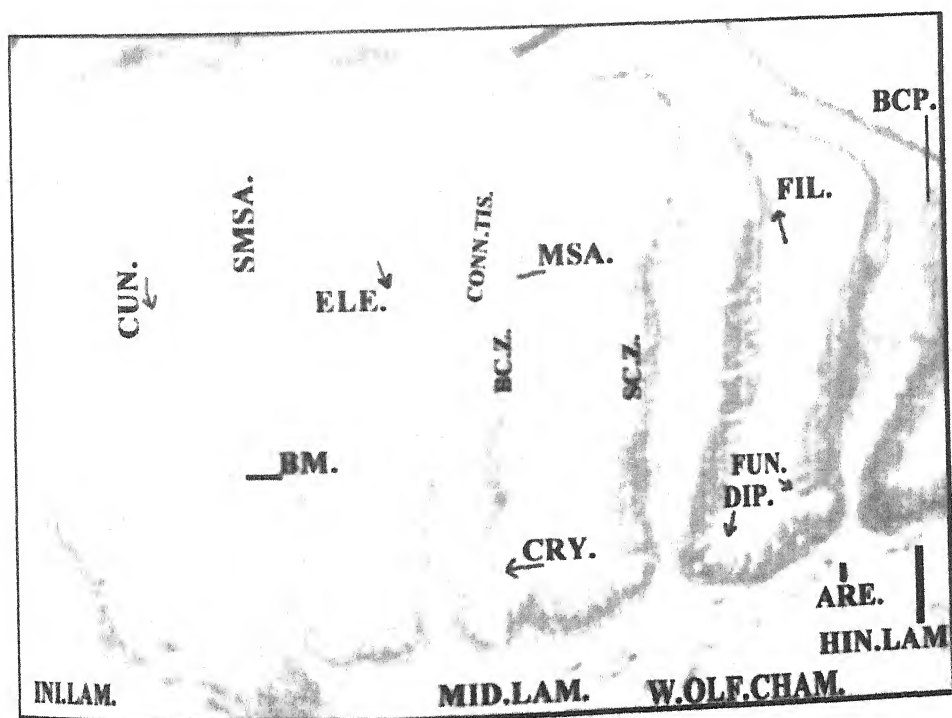


Plate.4

the bulbs. Behind the telencephalon lies the mesencephalon which mainly consists of large optic lobes (OP.LO., Fig.-2).

The olfactory rosette (ROS.) of *Tilapia mossambica* is quadrangular in shape which bears lamellae (LAM.) in the form of thickenings, coming out from the olfactory epithelium. The rosette is devoid of raphe and its endings are free from the lamellar thickenings. The arrangement of olfactory lamellae in the central part of the rosette gives a rough impression of petals of lotus (Fig.-1B, C). All the lamellae are attached with the floor of olfactory epithelium with their ventral surface and remain projected in the olfactory chamber through their dorsal surface. The lamellae are separated from each other by well defined interlamellar spaces (INT. LAM. SP.) whereas their distal and proximal ends remain attached with the rosette (Plates-3,4). The lamellae are constituted of central core or submucosa (SMSA.) lined on both the sides by cellular components of mucosa (MSA.). The mucosa is mainly constituted of ciliated columnar epithelium and abundantly supplied with basal cells (BC.). The basement membrane (BM.) stands as partition in between submucosa and mucosa. The mucosal zone exhibits great variation in its thickening in all the lamellae and possesses some peculiar microformations which are due to the flow of basal cells in different patterns. This flow causes the displacement of other cellular components and subsequently leads in the formation of cuneiform (CUN.), filiform (FIL.) and fungiform (FUN.) mucosal surface which is supposed to increase the olfactory area. With the result of these formations, there appears depressions (DIP.), elevations (ELE.) and crypts (CRY.) of different shapes and sizes (Plates-3,4,5,6,7,8).

Plate-5 : Magnified section of olfactory lamella of *T. mossambica* demonstrating the grouping of primary neurons in crypts, grouping of rod shaped receptors in depression forming olfactory receptive point, migratory basal cell groups showing offshoots to submucosa in the direction of microformations. Submucosa with dense connective tissue fibres, folium olfactorium, histocytes, mast cells, fibroblasts, areolae and grouping of transforming basal cells can be easily visible. Magnification 750X.

ARE.	-	Areolae
BM.	-	Basal membrane
BC.Z.	-	Basal zone
CRY.	-	Crypts
DN. P.N.	-	Dendrite of primary neuron
DN.R.R.	-	Dendrite of rod shaped receptor
FOL.OLF.	-	Folium olfactorium
FIB.	-	Fibroblast
GC.	-	Goblet cell
GR.PN.	-	Group of primary neurons
HIS.	-	Histocytes
MIG.GC.	-	Migratory goblet cell
MC.	-	Mast cell
NU.RR.	-	Nucleus of rod shaped receptor
RR.	-	Rod Shaped Receptor
SC.Z.	-	Supporting zone
SMSA.	-	Sub mucosa

Plate-6 : Magnified section of *T. mosambica* passing through the different types of crypts with the accumulation of primary neurons and their dendritic extension to the lumen of crypt. Olfactory cilia of rod shaped receptor is also visible projecting into the interlamellar space. Transitional basal cells in the preparation of their migration leading to subjective formation for the increase of receptive surface. Magnification 750 X.

ARE.	-	Areolae
BC.Z.	-	Basal zone
FIB.	-	Fibroblast
HIS.	-	Histocytes
INT.LAM.SP.	-	Inter lamellar space
MIG.GC.	-	Migratory goblet cell
MSA.	-	Mucosa
OCI.	-	Olfactory cilia
SMSA.	-	Sub mucosa
VAC.	-	Vacuole

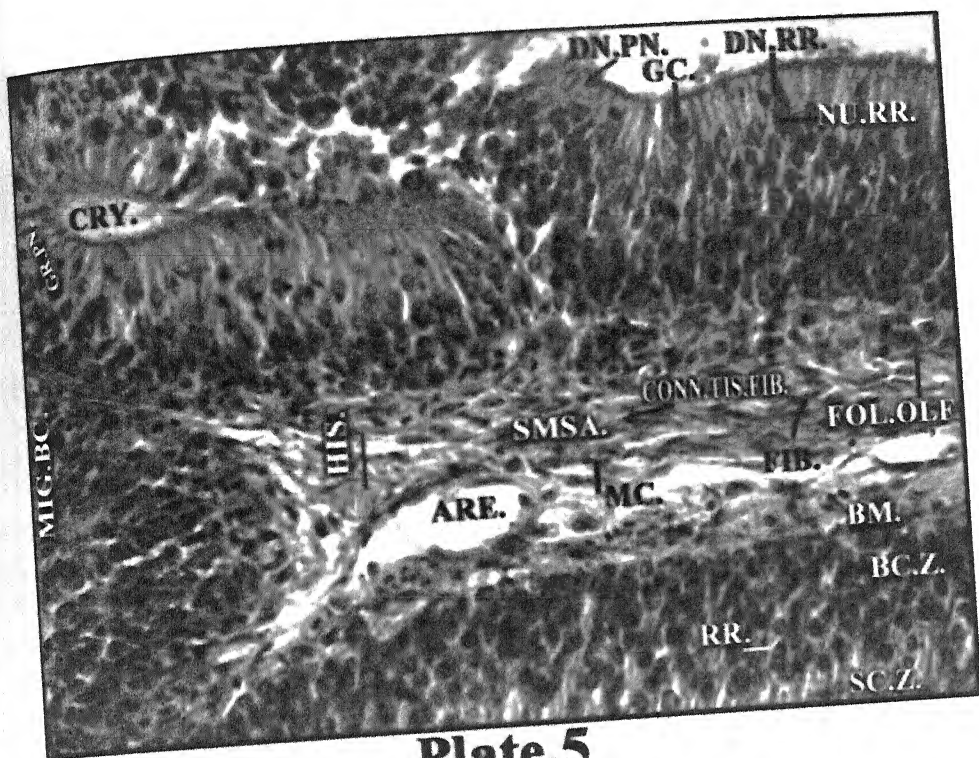


Plate.5

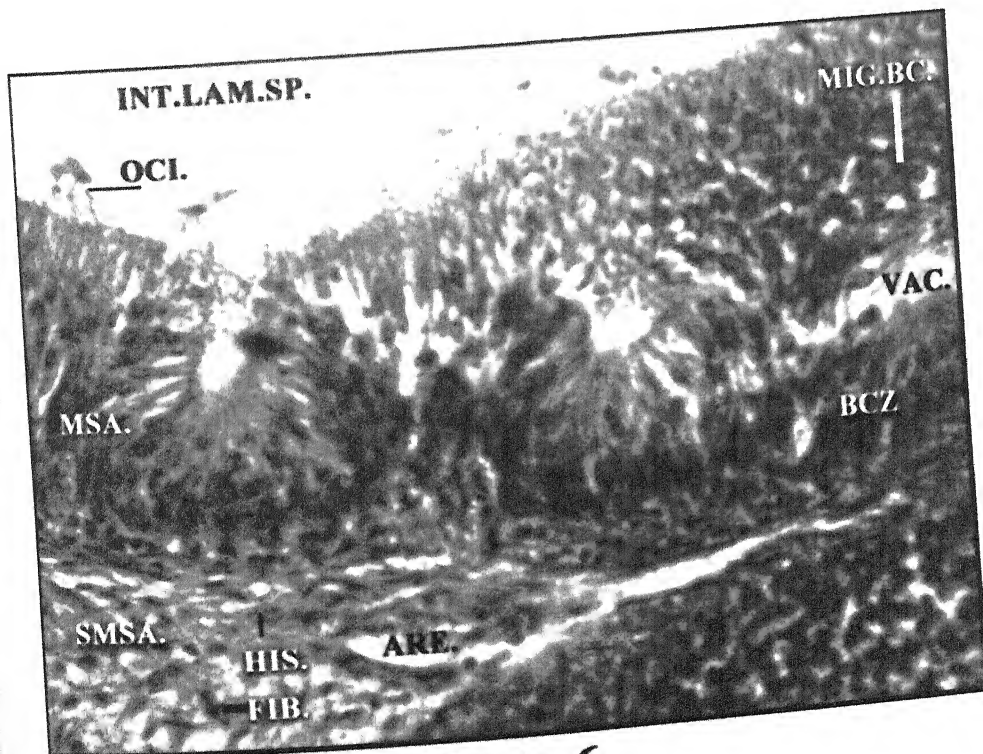


Plate.6

The grouping of basal cells and their migration (MIG.BC.) in different patterns in the surface of lamella causes the formation of "Crypts" (CRY.) of different shapes and sizes which are sunken in the lamellar surface at different depths. Such formations are richly supplied with primary neurons which, projecting their dendrites into the lumen interlamellar spaces (Plates-5, 6, 7). The minor lamella (MIN. LAM) are also observed but they are present in the interlamellar spaces of middle lamellae (MID. LAM.), formed of only mucosal cellular components. The microformations are richly visible in the middle and hinder lamellae whereas initial ones do not exhibit such features. It is commonly observed that the lamellae are subjected to the activity of basal cells which may push the mucosa in the form of bulging (Plate-4) at any place and giving the shape of transitionary epithelium, which may proceed in the direction forming microformations. The olfactory epithelium also discharges or extrudes its cells in groups or in solitary condition which can be observed in the interlamellar spaces. The broadening of submucosa is very prominent in the hinder lamellae and it widens at the expanse of mucosa, causing the reduction of later to a thin zone (Plate-3,4). From the submucosal point of view, the lamellae can be divided into three categories : (i) initial lamellae, (ii) middle lamellae and (iii) hinder lamellae.

The initial lamellae (INI. LAM.) are well composed having their terminal ends pointed whereas narrow at the base and broader in the middle part. These lamellae are provided with well built mucosa, made up of columnar ciliated supporting cells (SC.) and compactly built submucosa (Plates-4,9,11).

Plate-7 and 8 :

Magnified section of hinder lamellae of *T. mossambica* showing crypts, filiform, fungiform, cuneiform shapes along with transitionary basal zone. Submucosa contains blood, connective tissue, nervous, fibroblast, histocyte and mast cell supply. Grouping of rod shaped receptor cells in these said elevations is clearly visible. Magnification 750 X.





Plate.7

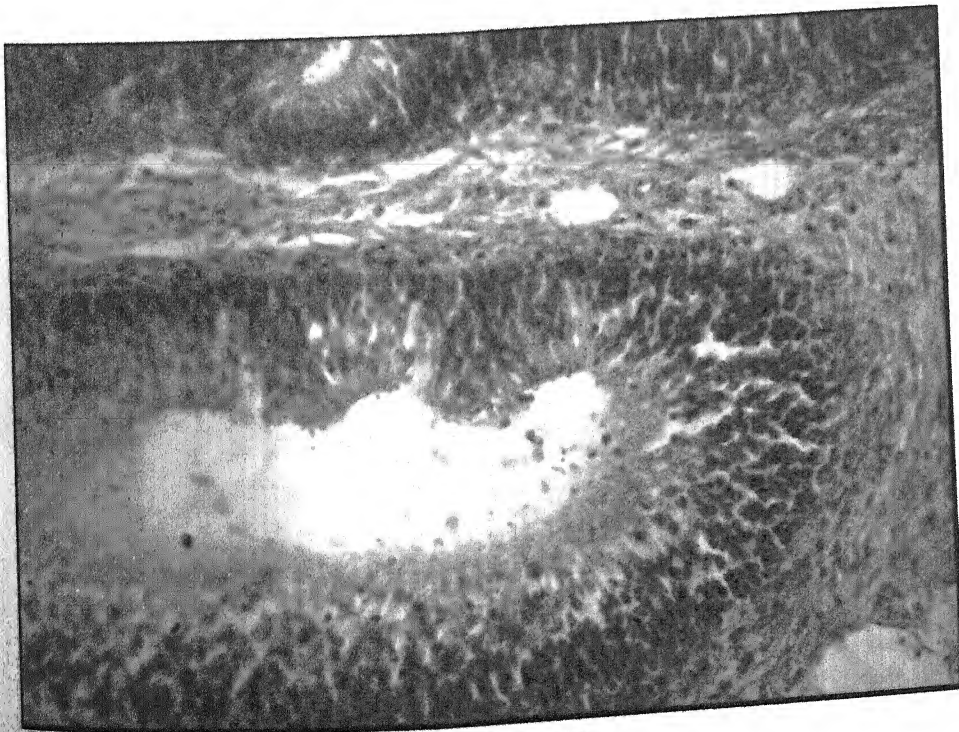


Plate.8

The middle lamellae are comparatively broader, having widening in the submucosa but not at the expanse of mucosal zone which remains well formed and shows scattered fibroblasts (FIB.) histocytes, (HIS.) and dense matrix entangled with connective tissue fibres (CONN. TIS.FIB., Plate-4).

The hinder lamellae are provided with enormously developed submucosa which causes the reduction in the thickening of mucosal zone. With the result of widening of submucosa, areolae (ARE.) appear due to the rare distribution of connective tissue fibres and other cells in the zone. The basement membrane (BM.) is pushed to the periphery. These are old and worn out lamellae which have attained full size (Plates-4, 7, 8).

The cellular components in *T. mossambica* may be identified as supporting cells, receptor cells, goblet cells and basal cells. The submucosa is supplied with connective tissue fibres, fibroblasts, histocytes, basal and pigment cells (PIG.C.).

Supporting Cells :

The supporting cells (SC.) of *T. mossambica* are subjected to great variation because of the enormous production of basal cells (BC.) and their subsequent migration in different patterns showing changes in mucosal region (Plates-5,6).

The supporting cells are ciliated and present in well composed initial lamellae. In the middle and proximal parts, these cells are having elongated body with oval nucleus which bear one or two nucleolus. The chromatin material is visible and distributed in karyoplasm. The outer or distal limbs are elongated, extending upto the peripheral surface of the lamellae which bear cilia. The cilia (CI.) of

supporting cells are considerably long, projected in interlamellar spaces and showing a trend of directional movement, depending upon the pressure of water coming out from both the accessory nasal sacs. The outer or distal limb of the supporting cell contains homogenous and eosinophilic cytoplasm. The inner or proximal limb is inconspicuous and difficult to trace among the other cellular components, lying beneath these cells.

With the result of great variation in mucosal surface, the supporting cells are affected and exhibit variation in their shape and occurrence. The mucosa may be affected either by enormous broadening of submucosal zone or by the movement of basal cells. In the former case, the supporting cell becomes oval and short with almost oval nucleus and invisible chromatin material. These cells become inconspicuously ciliated and bear short outer or distal limb. In the zone of micro formations where tremendous migration of basal cells is observed, the supporting cells cannot be clearly identified from migratory basal cells and receptor cells. Such zones which may be either in the form of elevations or depressions, the supporting cells are of the size of basal cells or may be in the formative stage, leading to microformations of different patterns (Plates-5,6,7,8). The indifferent epithelium where basal cells migrate in different patterns, is supposed as the transitionary phase and receptor cells can only be identified because of clear dendrites and axons, extending in their respective direction in the mucosal zone.

Receptor cells :

The receptor cells are observed throughout the epithelium of *T. mossambica* irrespective of their restriction in any particular region of

the lamella. However, they are concentrated in the mucosal deepenings and olfactory crypts, formed for the purpose of increasing the olfactory surface area. Such deepenings are alternated by elevations in the shape of cuneiform, filiform, fungiform, and simple elevations which are richly supplied with elongated bodied receptors known as rod shaped receptor cells. . The receptor cells in *T. mossombica* can be identified as primary neurons and rod shaped receptors.

The primary neurons are confined in the crypts of different patterns and rarely observed in the mucosa of middle and hinder lamellae but absent in initial ones which are having well composed mucosal zone (Plats-5,6,11,14). They bear rounded nucleus (NU.PN.) and send fibrilar dendrites (DN.PN.) to the peripheral surface. The dendrite is darkly stained and bears some form of cilia (Cl.) on its terminal end which project in the opening of different patterns. These receptor cells are situated away from the basement membrane roughly in the middle of mucosa or sometimes situated terminally in the mucosal zone. With the result of migration of basal cells, the primary neurons are pushed at different levels in the uniform or ununiform mucosa but the identity of dendritic extension is clearly visible because it acquires a dark stain. The trace of axon is visible but not as clearly as that of dendrite because in the lower region cellular components are compactly packed. The terminal tip of the dendrite is visible in the form of dark stained spot, the receptor vesicle, bearing olfactory villi or olfactory cilia (OCI.).

The rod shaped receptor cells (RR.) are common in occurrence in the well composed mucosa of all the lamellae and specially in the

zones where olfactory epithelium is activated to give rise to deepenings and elevations of different patterns (Plates-5,6,7,8,9,11). They are present almost in the middle lower zone of mucosa and possesses oval, darkly stained nucleus (NU.RR.) with conspicuous dendritic (DN.RR.) extension towards the interlamellar spaces. The terminal tips of dendrites bear some form of cilia, projected in the interlamellar spaces. The axonal extensions of these rod shaped receptor cells can be clearly traced out. These receptors are present almost at the level below the zone of supporting cells, thereby having less elongated axons which sometimes give the appearance that the rod shaped receptors are directly coming out from folium olfactorium (FOL.OLF).

The synaptic (SYN.) contact between any two receptor cells has not been observed anywhere in the olfactory epithelium of *T. mossambica* and independent identify of each type of receptor cell is maintained in both solitary and aggregatory arrangement. The axons of all the receptor cells extend proximally and join folium olfactorium along the basement membrane.

Goblet cells :

The goblet cells (GC.) are rare in occurrence and occasionally observed in different zones of mucosal layer. They can be rarely seen at any level of mucosa and originate from the basal zone due to muciferous activity of basal cells. The mucous cells are richly supplied in the epithelium of accessory nasal sacs (ACC. NAS. SAC., Plates-12,13). Because of rare occurrence of goblet cells, very little muciferous activity is observed in the olfactory rosette of *T. mossambica*. The observation of goblet cells at different levels in the mucosa from basal to supporting zone, demonstrates that the muciferous basal cells are

created in the basal zone which migrate upto the peripheral region for the discharge of their mucous into the interlamellar space. The goblet cells possess rounded to elongated body. They gradually grow in size and exhibit muciferous activity as they come to peripheral surface, where the terminal tips of theca of goblet cells project in the interlamellar spaces for easy discharge of the mucous. The goblet cell bears round to elongate theca (GC.TH.) with triangular nucleus, which can be deeply stained with haemotoxylin and shows its inconspicuous stalk upto the basement membrane. The chromatin material and nucleus is not visible. Though the muciferous activity is very restricted in *T. mossambica* because of rare occurrence of goblet cells, however, deposition of mucous in the histological sections in present investigation has been observed on the peripheral surface of the lamellae.

Basal cells :

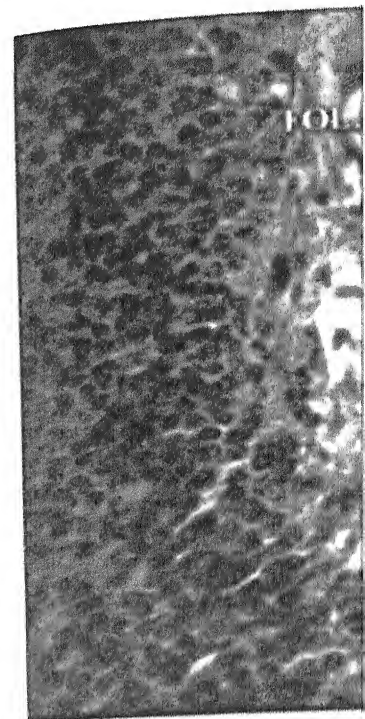
The basal cells (BC.) can be distinguished in a number of forms, lying regularly or irregularly above the basement membrane and contributing a major part of the mucosa. Each basal cell is provided with a darkly stained oval nucleus (NU.BC.) with centrally placed nucleolus and uniformly distributed chromatin material. The basal cells can be seen both in mucosa and submucosa and can be identified by their rounded shape and darkly stained nuclei. In some zones of mucosa the basal cells exhibit a tremendous tendency of migration, leading to different patterns of elevations and deepenings. Both these shapes are accumulated result of the large production of basal cells due to cell division and their subsequent flow in any direction, resulting unmanageable aggregation of mucosa which can

Plate-9 : Magnified section of proximal end of 1st lamella of *T. mossambica* showing uniform surface with dense distribution of basal cells, ciliated and nonciliated supporting cells, rod shaped receptor cell, basement membrane, folium olfactorium and all elements of submucosa is clearly visible. Magnification 750 X.

BC.Z.	-	Basal zone
FOL.OLF.	-	Folium olfactorium
PRO.E.LAM.	-	Proximal end of lamella
RR.	-	Rod shaped receptor
SC.Z.	-	Supporting zone
SMSA.	-	Sub mucosa

Plate-10 : Magnified demonstration of submucosa of olfactory chamber of *T. mossambica* showing stellate type of chromatophores, blood capillaries, collagen connective tissue fibres, histocytes, fibroblasts, mast cells, areolae, nervous supply and lymphocytes. Magnification 750 X.

HIS.	-	Histocytes
PIG.	-	Pigment cell
SMSA.	-	Sub mucosa



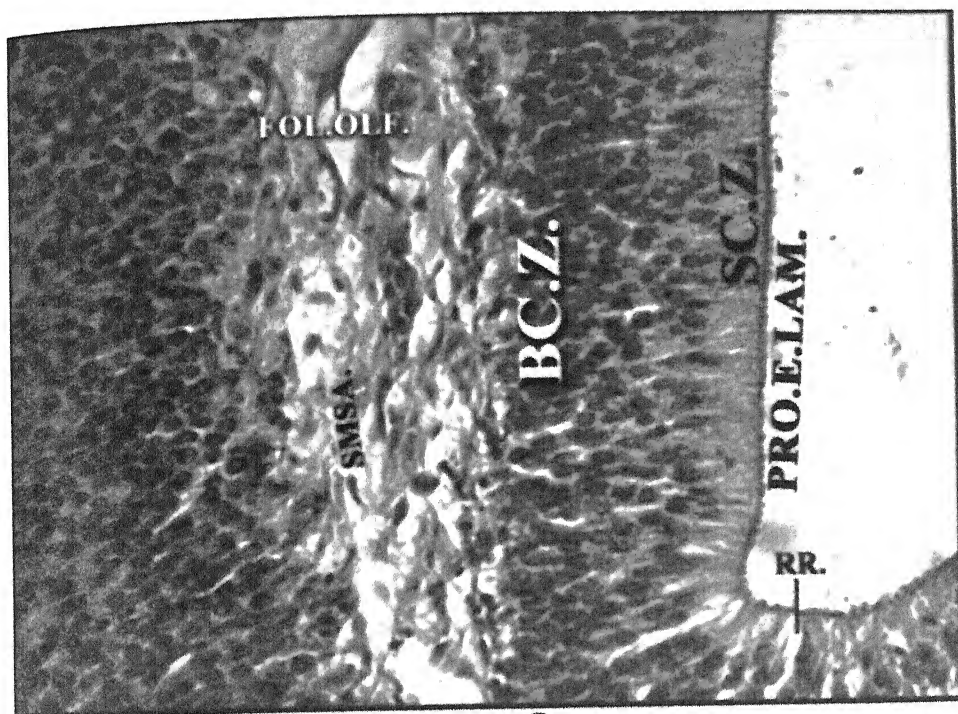


Plate.9

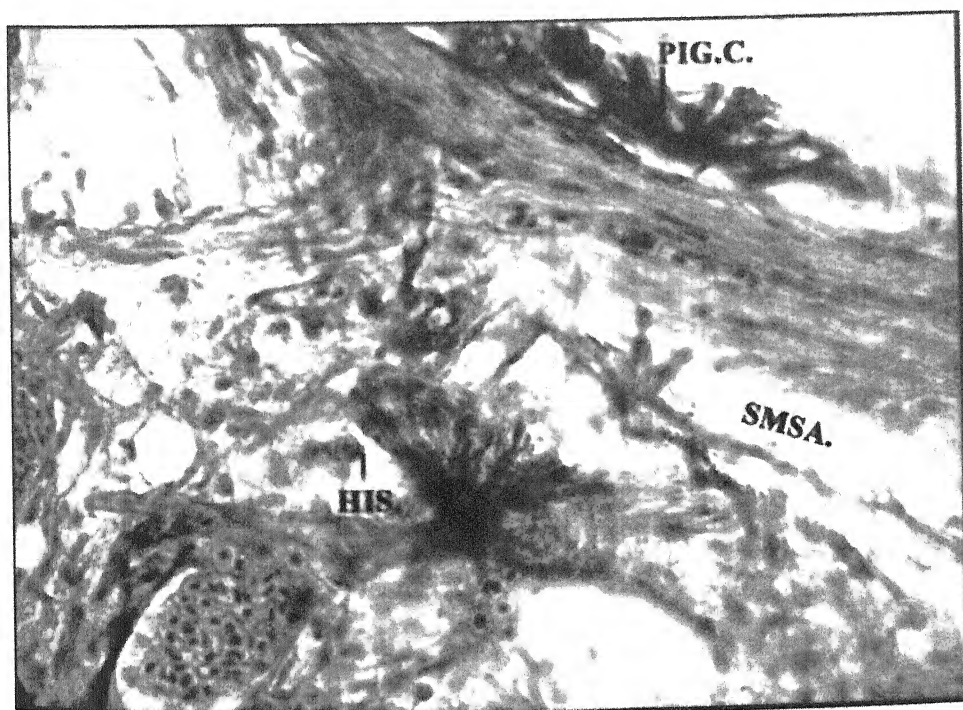


Plate.10

be named as indifferent or transitional mucosa. (Plates-5,6,7,8,14) Such transitional or indifferent mucosa becomes regularised in its form after taking the shape of deepenings and elevations. The former may be in the form of flask, vacuole, funnel, tubule etc., whereas the later in the shape of cuneiform, filiform, fungiform simple and major elevations. In such places submucosa becomes more activated and plays its role in supplying nutritional contents through blood circulation, so as to nourish the large production of basal cells and their flow properly. The purpose of increase of olfactory surface in different patterns may be served successfully for the proper discharge of olfactory and other functions related to olfactory epithelium.

The flow of basal cells occurs in well fed lamellae and as soon as the flow is started or prior to initiation of flow, the mucosal surface acquires the form of transitional epithelium which in due course of time converted into formations described earlier. It is rarely observed that minor lamella is also a result of the flow of basal cells from the olfactory epithelium which is devoid of central core or submucosa. The minor lamella is compactly formed and it is the outpushing of mucosal zone. During the course of flow of basal cells and the formation of deepenings and elevations, the basal cells are extruded out in the interlamellar spaces which may be washed away with circulatory water current.

Central core or submucosa :

The central core or submucosa (SMSA.) is greatly varied in its composition and widening in different lamella of *T. mossambica*. The submucosa does not extend in microformations and elevations because these are solely made up of mucosal cellular components.

Plate-11 : Magnified section of distal narrow end of lamellae of *T. mossambica* showing vacuole like crypt, narrow submucosa with narrow basal and supporting zone. Rod shaped receptors are present at free surface while primary neurons are accumulated in crypts at deeper zone of mucosa. Magnification 450 X.

BC.Z.	-	Basal zone
CRY.	-	Crypts
DIS.E. LAM.	-	Distal end of lamella
SC.Z.	-	Supporting zone
SMSA.	-	Sub mucosa

Plate-12 : Magnified section passing through lacrymal accessory nasal sac of *T. mossambica* showing narrow submucosa with thickly distributed elastic connective tissue fibres. Mucous filled beaked goblet cells, cuboidal supporting cells and basal cells are distributed in thin zones. Sac space is also visible. Magnification 750 X.

LAC.ACC.NAS.SAC.	-	lacrymal accessory nasal sac
SMSA.	-	Sub mucosa



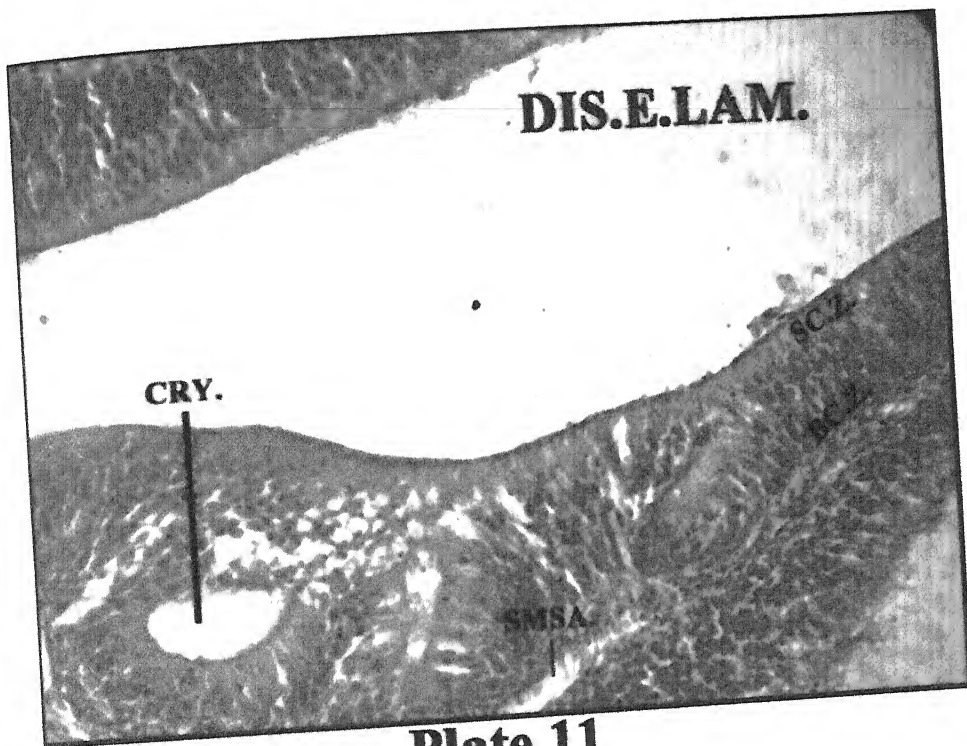


Plate.11

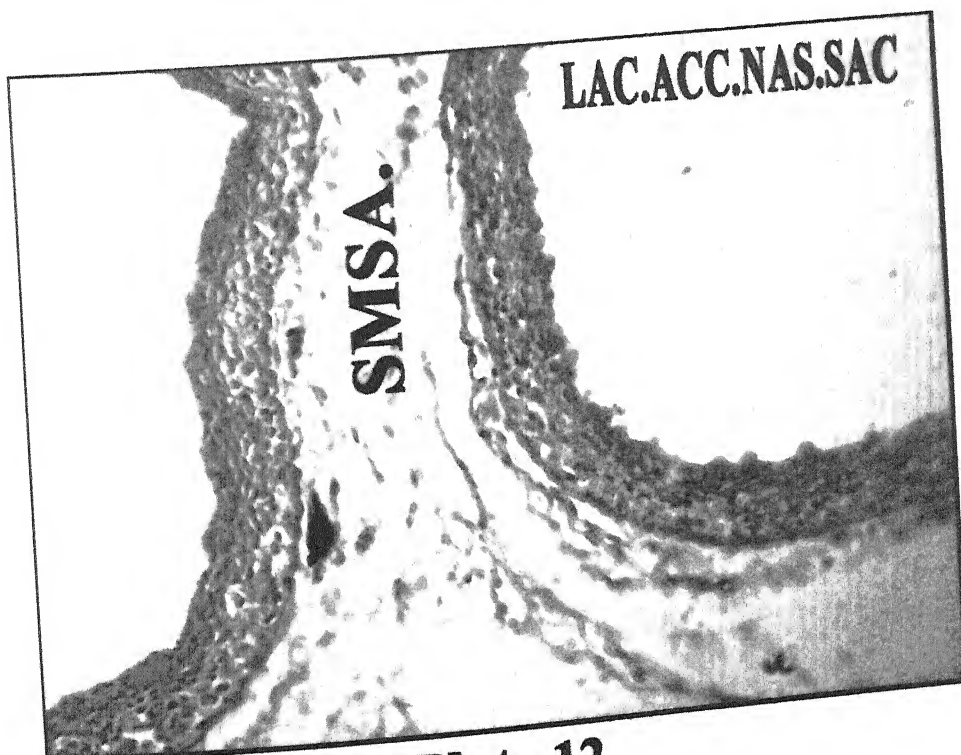


Plate.12

Plate-13 : Magnified section passing through ethmoidal sac of *T. mossambica* showing hillock elevation, mucous filled goblet cells, cuboidal supporting cells, thick basal zone and submucosa with elastic connective tissue and other cellular contents are clearly visible. Magnification 750 X.

- | | |
|----------------|-----------------------------------|
| ELS.CONN. TIS. | - Elastic connective tissue |
| DN. RR. | - Dendrite of rod shaped receptor |
| GC. | - Goblet cell |
| HIL. ELE. | - Hillock Elevation |
| SMSA. | - Sub mucosa |

Plate-14 : Magnified section of rosette of *T. mossambica* passing through the proximal region depicting crypt and deeper vacuole like microformation with cuneiform, filiform and fungiform formations with the grouping of primary neurons sending their dendrites in crypts and vacuole lumen for olfactory reception. In other parts rare occurrence of goblet cells and rod shaped receptors are visible. In funiform, fungiform and cuneiform forms rod shaped receptors, ciliated and non ciliated supporting cells, mass of basal cells in basal zone are visible. Structure of submucosa and mucosa with their connective tissue fibres, fibroblasts, mast cells, histocytes is clearly demonstrated.

- | | |
|-------|--------------|
| CRY. | - Crypts |
| SMSA. | - Sub mucosa |
| VAC. | - Vacuole |



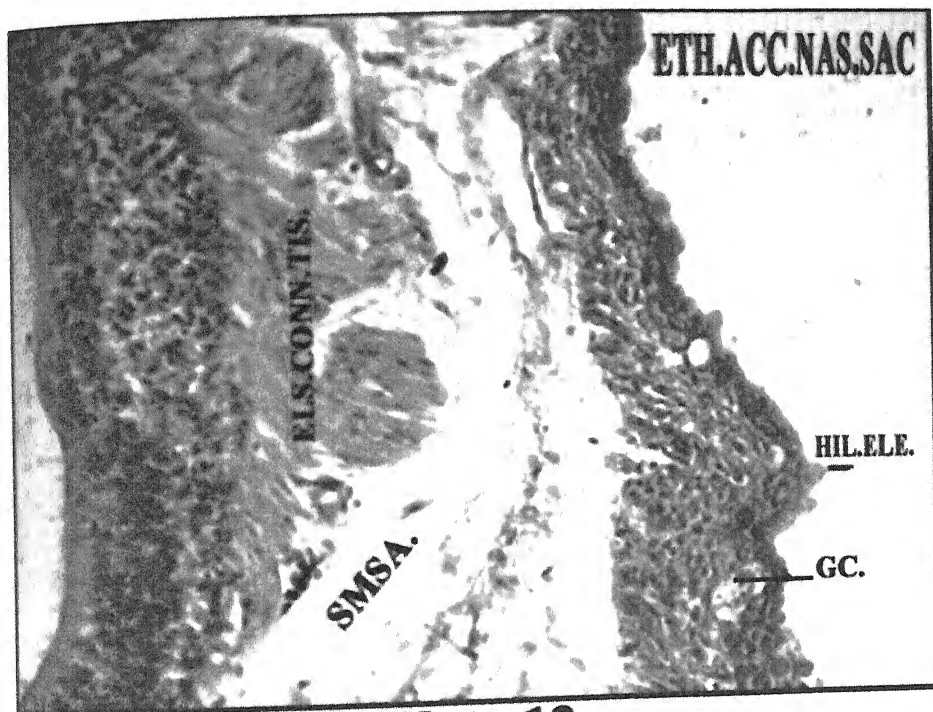


Plate.13

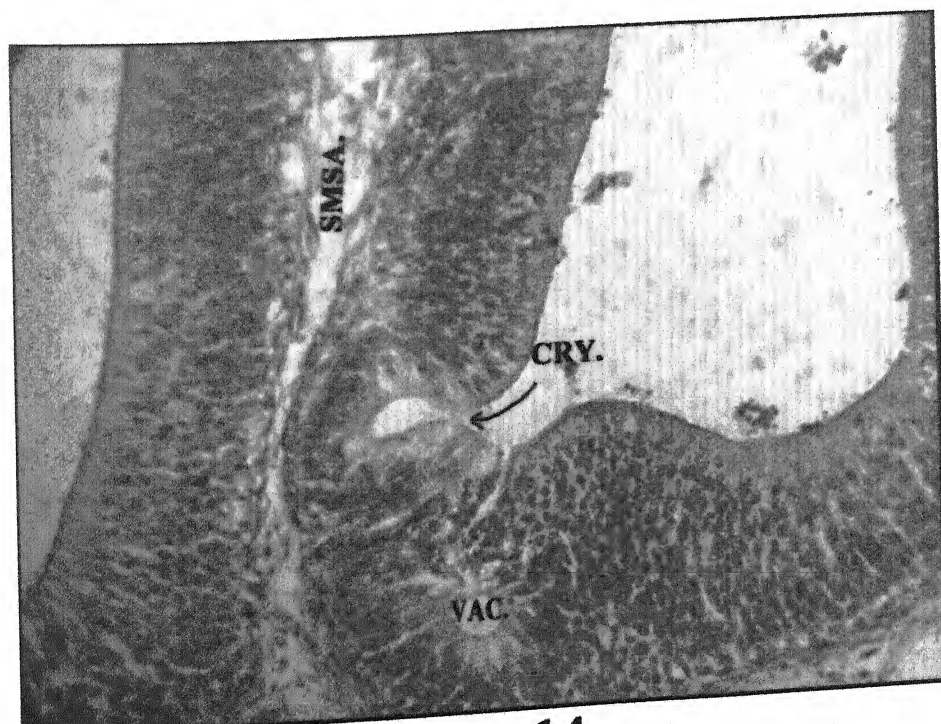


Plate.14

The matrix of submucosa is formed by connective tissue fibres (CONN.TIS.FIB.) with dense supply of fibroblasts (FIB.), histocytes (HIS.) mast cells (MC.) areola (ARE.) and basal cells (Plates-5,6,7,8,10,14). The pigment cells (PIG.) are concentrated in the olfactory rosette at the point of emergence of olfactory lamella and the surrounding of blood sinuses (Plate-14). The nonmedullated nerve fibres (NMN.FIB.) run below the basement membrane which join medullated nerve fibre bundles (MN.FIB.) at the point of emergence of lamella in the olfactory rosette. Branched fibroblasts and shapeless histocytes are common in occurrence in the submucosa. In the initial lamellae, the submucosa is supplied with connective tissue fibres, having properly distributed fibroblasts, histocytes and basal cells (Plate-4,9). As we proceed to hinder region of the rosette, the submucosa grows abnormally, pushing the mucosa to a thin layer (Plates-7, 8). In such cases connective tissue fibres becomes irregularly distributed having a rich supply of fibroblasts, histocytes and basal cells. Areolae are present in broadest submucosa. The blood capillaries (BCP.) and nonmedullated nerve fibre bundles are observed in the submucosa of all the lamellae. The connective tissue fibres provide support to the lamellae. Specific turgor formation for strengthening the lamellae, is not observed in *T. mossambica*.

Accessory nasal sacs :

The ethmoidal and lacrymal accessory nasal sacs of *T. mossambica* are made up of nonciliated cuboidal epithelium (Plates-13,14). The epithelial lining of the sacs is wavy and shows elevations and depressions (Plate-13). It is constituted of cuboidal supporting cells, rounded goblet cells and basal cells.

The cuboidal cells are situated in the periphery with darkly stained oval nuclei. They can be seen in two or three rows in elevated regions of the epithelium. The goblet cells are rounded, neckless and found embedded in the peripheral epithelial surface. They can also be observed with empty theca after discharging their mucous. The goblet cells may also be present in two or three rows in the regions of elevations. The basal cells are lying in three or four rows just above the basement membrane. In elevations, the basal cells are accumulated in large number, showing their migratory tendency towards the periphery (Plate-13).

The wavy basement membrane lies just below the basal cells. The connective tissue fibres are loosely arranged in the submucosa. The fibroblasts and basal cells are intermingled with connective tissue fibres. The blood capillaries are also present in the submucosa of accessory nasal sacs.

The number of sac layer varies with the distension of accessory sac. In normal condition, the cuboidal epithelial and basal cells are accumulated in seven to eight layers. The connective tissue fibres and basement membrane are wavy (Plate-13), however, in distended condition the accessory sac consists of 2-3 layers of basal cells and the basement membrane and connective tissue fibres are stretched (Plate-14). Both the ethmoidal and lacrymal accessory nasal sacs exhibit similar histological picture in the present investigation.

Ecological Coefficient :

The ecological coefficient in *T. mossambica* measuring from 115 to 176mm total length, was calculated by two methods :

- (i) by taking the length of mesencephalon and telecephalon as parameter and
- (ii) by measuring the areas of two retinae and both the rosettes. By comparing the former with that of latter, the effectiveness of optic and olfactory faculties can approximately be assessed.

By comparing the former with that of later, the effectiveness of optic and olfactory faculties can approximately be assessed. The length of mesencephalon and telencepholon varies from 2.936 to 3.510mm and 2.053 to 2.340mm respectively, showing the ecological coefficient range from 65.847 to 69.925% (Table-7)

From the area point of view, both the rosette varies from 39-934 to 70-113mm² whereas those of two retinae ranges from 72.827 to 80.206% (Table-7).

The results thus obtained show that *T. mossambica* is a microsmatic fish because it possesses tremendously developed optic faculty as compared to that of olfactory faculty which is very much regressed. This suits to its highly predaceous habit, preying on fishes half of its size. In this action *T. mossambica* visually target the fish and capture the same as its prey.

Route of water circulation through the olfactory chamber of *T. mossambica* :-

The fish exhibits a characteristic of continuous protruding and retracting its jaw apparatus during normal swimming and feeding conditions. The above action, in addition to forward movement of fish, causes the entry of water through anterior nasal opening. The water circulates in the olfactory chamber freely, as its major part is

lamellaeless and whatever the lamellae present are the simple thickenings of the floor of olfactory chamber. It is connected with the ethmoidal and lacrymal accessory nasal sacs by well defined openings. Immediately after reaching into the olfactory chamber through anterior nasal opening, water takes its route to both the accessory nasal sacs through their separate openings. When the mouth is closed, jaws are retracted causing the reduction in the volume of accessory nasal sacs and olfactory chamber. This leads to the expulsion of water from posterior nasal opening. The valve condition of posterior nasal opening demonstrates unidirectional flow of water from anterior to posterior nasal opening (Fig. 1A, B, C).

Table-7 : Ecological coefficient of *T. mossambica*

Sl. No.	Total length (mm)	Number of lamellae		Total length of brain (mm)	Length of mesencephalon (mm)	Length of telencephalon (mm)	Ecological coefficient (through lobes of brain) Length of telecephalon x 100 Length of mesencephalon	Retinal area of both eyes (mm ²)	Olfactory area of both rosette (mm ²)	Ecological coefficient (through area) Olfactory area x 100 Retinal area
		Right	Left							
1.	115	7	7	8.775	2.936	2.053	69.925	54.834	39.934	72.827
2.	127	7	8	9.243	3.159	2.094	66.286	58.647	44.237	75.429
3.	133	8	8	9.594	3.217	2.117	65.847	61.524	49.346	80.206
4.	142	8	8	10.620	3.274	2.223	67.898	70.986	55.053	77.554
5.	176	10	9	11.700	3.510	2.340	66.666	90.832	70.113	77.189

Histochemical observations of olfactory epithelium of *Tilapia mossambica*.

Acid Phosphatase :

The acid phosphatase activity in *Tilapia mossambica* is relatively of high degree along cell and nuclear membrane of various types. It is specifically more in the dendrites of primary neurons, distal extremity of columnar supporting cells. It is also observed in cytoplasm and some granules present in cytoplasm which acquires dominant stain. These granules which are probably lysosomes, that are supposed as the pooling cell organelles of hydrolytic enzyme in *T. mossambica*. These granules (Pysosomes) make available the metabolite to the cell concern for the production of energy and elsewhere utilization of metabolites for the other requirement of cells such as growth, repair and resistance to fight against infection.

Tilapia mossambica is an exotic fish and is subjected to number of climatic variations in the route of its adaptability for acclimatization in a particular captive environment. The lysosomes contains hydrolytic enzymes, whose marker is acid phosphatase and therefore it reveals intense activity in cell sites (Table-1). For confirming this concept, acid phosphatase activity in *T. mossambica* is higher in columnar supporting cells, dendrites, olfactory cilia and in some basal cells who are in active mitotic division in the preparation of their route either for their transformation in some other cell type or for fulfilling the requirement of olfactory epithelium under the conditions of injuries and creation of microformations (Table-1).

Alkaline Phosphatase :

Alkaline phosphatase is seen remarkable higher in basal zone and at interlamellar level. The activity in basal cells present in submucosa or central core is also higher in *T. mossambica*. The alkaline phosphatase is a supporting precursor for acid phosphatase in the process of providing metabolite to the cell for the purpose of their utilization under the influence of acid phosphatase. *T. mossambica* is a rafeless fish and fewer lamellae are seen originating from peripheral wall of olfactory chamber and converging to the central. This indicate that lamellae in the centre region of rosette are in growing phase and activity of alkaline phosphatase in such tips is intense. These tips are having indifferent olfactory mucosa, where few receptors and goblet cells can only be identified. The lymph space at the emergence of lamellae through peripheral olfactory wall also contain dense connective tissue system. In such tissues basal cell, fibroblasts and other cells related with supporting and nutrition also show alkaline phosphatase activity. In the region of lamellae through which nerve are innervated and pass to the ultimate regions through folium olfactorium and non medulated nerve fibres also show aggregation of some cells where in alkaline phosphatase activity is visible (Table-2).

Glycogen :-

It is a general concept that glycogen is a reserve potential energy, which after converting into glucose (passing through a metabolic cycle) release chemical energy required for the building of ATP. The cell structure in the olfactory epithelium of *T. mussambica* which are engaged in greater activity shows high degree of localisation

of glycogen. The supporting cells with their cilia and nucleus shows amountable localization of glycogen. The primary neurons as compared to rod shaped receptor cells shows dominant glycogen activity. The glycogen deposition in the basal cells lying exactly along the basement membrane is greater, which is supposed as their preparation in the process of forming other cell type for the growth of lamella and in the increase of olfactory reception surface. This takes place by the way of rapid mitotic division in such cells which requires energy and is obtained by reserve glycogen.

Goblet cells are the unicellular gland which are constantly discharging mucous content for protecting the olfactory mucosa from soft and other damages of circulating water current. These cells are short lived but subjected to higher metabolic activity. The secretory content is also constituted of major bulk of glycogen reserve (Table-3).

Acid Mucopolysaccharides :

The localization of acid mucopolysaccharides is restricted to the goblet cell as it is the main content in the constitution of mucin in *T. mossambica*. However, its demonstration also occur in some other cell types in supporting and basal zones. This indicate that muciferous activity are gradually cultivating in basal and supporting cells which may ultimately grow to full grown goblet cell. Thus, cells exhibiting the demonstration of acid mucopolysaccharides in basal and supporting zone are identified as transitional basal and supporting cells which are in the process of their conversion to goblet cells (Table-4).

Lipid :

Lipid localization in *T. mossambica* is conducted in an usual manner. It is observed that the distal tip of the lamella has moderate

concentration of lipid in their supporting cells but in proximal zone of lamellae, the concentration of lipid in supporting cells increases in greater degree. Remarkable observation in this regard is that, the lipid core is at high degree in the basal cell above the basement membrane but in such cells where mitotic activity is reported the lipid core is in negative degree. At the point of convergence of all the lamella in centre, adipose tissues are visible which has got high conc. of lipid deposition. In receptor cell and dendrite projections lipid conc. is moderate, but where the axons join to folium olfactorium, concentration is intense (Table 5).

Metacromasia :

The olfactory epithelium of *T. mossambica* exhibit positive metacromasia demonstration in all the cellular extension of all mucosal elements. It is demonstrated in axon and dendrite, including folium olfactorium and non medulated nerve fibre bundle present in the central girdle of *T. mossambica*. The stock of mucous secretory goblet cells also reveals feeble reaction of this histochemical contents. The limbs of different type of receptor cells, supporting cells, basal cells demonstrates moderate degree of localization. Its representation in cytoplasm indicates the granulation in it. Peripheral surface of entire lamella react negatively in response to toluidine blue (Table -6).

Table-1 : Showing histochemical distribution of **Acid Phosphatase** employed by Pearse 1968, and the reaction obtained in various cellular components of olfactory epithelium of *Tilapia mossambica*..

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
Acid Phosphatase	Cryostat	Modified Lead nitrate method Processed as recommended by TAKEUCHI and TANOUE as given by Pearse, 1968.	(i) Primary neurons	+++ (dendrite)	+++ = High activity
			(ii) Rod shaped receptors cells	++	++ = Moderate activity
			(iii) Columnar supporting cell	++ (distal limb) ++ (cilia)	+ = Low activity - = Absence of any activity
			(iv) Basal cell	+++	
			(v) Goblet cell	++	

Table-2 : Showing the demonstration of **Alkaline phosphatase** employed by Pearse 1968, and the reaction obtained in various cellular components of olfactory epithelium of *Tilapia mossambica*.

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
ALKALINE PHOSPHATE	Cryostat	Calcium-Cobalt method (After GOMORI, as given by PEARSE, 1968).	(i) Primary neurons	+	+++ = High activity
			(ii) Rod shaped receptor cells	+	++ = Moderate activity
			(iii) Columnar supporting cell	-	+ = Low activity
			(iv) Goblet cell	-	- = absence of any activity
			(v) Basal cell	+++ (basal zone, sub mucosa and connective tissue)	

Table-3 : Showing the histochemical location of **Glycogen** in olfactory epithelium of *Tilapia mossambica*

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
Glycogen	Microtomy, 8 μ m	Periodic Acid schiff technique and Best Carmine stain	(i) Primary neurons	+++	+++ = High activity
			(ii) Rod shaped receptor cells	++	++ = Moderate activity
			(iii) Columnar supporting cell	+++ (Cilia, nucleus)	+ = Low activity
			(iv) Goblet cell	+++	- = absence of any activity
			(v) Basal cell	++ (Basal Zone)	

Table-4 : Showing the histochemical localization of *Acid mucopolysaccharides* in the various cellular components of olfactory epithelium of *Tilapia mosambica*.

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
Acid mucopolysaccharides	Microtomy 8 μ	Alcianblue method [after STEEDMAN, vide PEARSE, 1968]. Deposition showing the Bluishgreen stains with Alcian Blue.	(i) Primary neurons	-	+++ = High activity
			(ii) Rod shaped receptor cells	-	++ = Moderate activity + = Low activity
			(iii) Columnar supporting cell	++	- = absence of any activity
			(iv) Goblet cell	+++	
			(v) Basal cell	++	

Table-5 : Showing histochemical technique for demonstration of *lipid* in the olfactory epithelium of *Tilapia mosambica*.

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
LIPID	Microtomy (temporary mount) 6µm	Sudan Black B method (after Mc MANUS Vide PEARSE, 1968)	(i) Primary neurons	++ (dendrite)	+++ = High activity
				+++ (axon joining folium olfactorium)	++ = Moderate activity
			(ii) Rod shaped receptor cells	++ (dendrite)	+ = Low activity
			(iii) Columnar supporting cell	+++ (proximal limb) ++ (distal limb)	- = absence of any activity
			(iv) Goblet cell	++	
			(v) Basal cell	+++	

Table-6 : Showing histochemical demonstration of *metachromasia* employed by pearse 1968, and the reaction obtained in various cellular components of olfactory epithelium of *Tilapia mossambica*.

ENZYME	SECTION	TECHNIQUE	CELLULAR COMPONENT	NATURE OF REACTION	REMARKS
Metachromasia	Microtomy (6 to 8µm)	Touildine Blue method (after KRAMER and WINDRUM, as given by PEARSE 1968)	(i) Primary neurons	++	+++ = High activity
			(ii) Rod shaped receptor cells	++	++ = Moderate activity + = Low activity
			(iii) Columnar supporting cell	++	- = absence of any activity
			(iv) Goblet cell	+	
			(v) Basal cell	++	

Chapter-4

Histological Discussion

Histological Discussion

Nasal Openings :

The olfactory chamber in the fishes are communicated to water by a pair of nasal openings, which are used for transportation of water through the olfactory chamber and not for breathing unlike in higher vertebrates. In teleosts a pair of nasal opening is present in each olfactory chamber and are named as anterior inlet and posterior outlet nasal opening. The two nostrils are so adjusted that one serves to intake the water and other for its exit (Allison, 1953; Lagler *et al.* 1962; Malyukina *et al.*, 1969 and Hara, 1975). According to Bateson (1889), Burne (1909), Teichmann (1954), Kleerekoper (1969), Hara (1975), Sharma (1981), Yadav (1989), Dubey (1991) and Sharma (2002), the olfactory chamber of most of the bony fishes bears two nasal openings which shows considerable variation in their shape, size and location in different fishes. In some fishes, anterior nasal opening is widely separated from the posterior while in others they lie close to each other.

Pipping (1926), Leirmann (1933) Lagler *et al.* (1962), Norman (1963), Gupta and Srivastava (1973) and Singh (1972) has reported single nasal opening in the olfactory chamber of some fishes. According to Burne (1909), the presence of single nasal opening may be the condition created by the elevation of the floor and subsequent rupture of the bridge between the nostrils.

In the present study of *Cyprinus carpio*, *Bagarius bagarius* and *Tilapia mossambica*, the olfactory chamber of all the three fishes bears

an incurrent anterior and excurrent posterior nasal openings. Bateson (1889) and Kapoor and Ojha (1973 b) advocated, that, the presence of anterior tubular nasal opening is characteristic of fishes having predominantly developed olfactory faculty. Kapoor and Ojha (1972 a and 1973 b) reported, that, when anterior and posterior nasal openings are separated from each other by some distance, then the former is invariably born on a tube. The presence of well defined tubular anterior nasal opening in *B. bagarius* is in accordance with the idea of Bateson (1889), Kapoor and Ojha (1972 a and 1973 b), because this fish has a well developed olfactory faculty. In *T. mossambica* and *C. carpio* anterior and posterior nasal openings are very closely situated while in *B. bagarius* the distance between the two nasal openings is moderate and both are found situated at two extremes of the head. The tubular anterior nasal opening in *B. bagarius* overhangs on the upper lip and projects in forward direction from the surface of the head. In *C. carpio* and *T. mossambica* nontubular anterior nasal opening is present which is thickly rimmed and situated close of the posterior nasal opening.

In *C. Carpio* the anterior nasal opening lies on a distinctly formed lip, whose hinder end is continued in the form of a forwardly and outwardly projected hood like nasal flap. This acts as a partition in between the anterior and posterior nasal opening and deflects the water to the olfactory chamber through anterior nasal opening. Branson (1963) reported in *Hybopsis gelida* and *H. aestivalis* that anterior nasal opening lies on a slight protuberance and partitioned from the posterior nasal opening by a nasal flap. The nostrils and nasal flap in *Cyprinus carpio* is in accordance with the Burne's (1909)

nostrils column IV. According to Burne (1909) and Teichmann (1954) the nasal flap is concave anteriorly, apparently serving to deflect water current downward into the anterior nostril, a rather general arrangement in bony fishes. But according to present investigation the presence of nasal flap is confined to cyprinidae and not a general arrangement in other bony fishes.

The nasal flap in *C. carpio* dips into the olfactory chamber by a curtain like extension from its ventral side and divide the chamber into anterior and posterior compartments. Similar curtain like extension of nasal flap is noted by Branson (1963) and Ojha and Kapoor (1973a).

The posterior nasal opening are of various shapes and they may be circular, oval, bean shaped, crescentic or rectangular. Burne (1909) reported that the size and shape of the posterior nasal opening vary significantly in different species. In *C. carpio* and *T. mossambica*, the posterior nasal opening are considerably larger than the anterior and are wide, covering most of the part of olfactory chamber and lamellae can be peeped through it. Branson (1963) reported considerable wide posterior nasal opening in *H. gelida* and *H. aestivalis*. The posterior nasal opening in *B. bagarius* is oval and is surrounded by collar-shaped fold of skin having a median notch. The nasal flap closes the opening and acts as a valve. Vulvular posterior opening have been described in several fishes (Bateson, 1889; Burne, 1909; Liermann 1933; Gooding, 1963; Kapoor and Ojha, 1972, 1973 b; Ojha and Kapoor, 1972; Rahmani and Khan, 1977; Sharma, 1981). The posterior opening of *B. bagarius* may be classified in Burne's (1909)

nostril column V. *C. carpio* and *T. mossambica* bears circular and bean shaped posterior nasal openings respectively.

The present author is of the opinion, that, in most of the fishes anterior nasal opening is situated above the surface of head either in the form of a tube or thickened margin or lips or on some protuberans, but posterior nasal opening is generally flush with surface of the skin. This may be a device for incurrent anterior nasal opening for making easy flow of water through the olfactory chamber from anterior to posterior nasal openings. The placement of the anterior nasal opening above the surface of the head helps in the entry of water current during the forward progression of the fish and similarly the flushed posterior nasal opening allowing the exit of same current without any hinderance.

According to Doving and Thommesen (1977), the olfactory passage in fishes is divided into anterior vestibule and posterior gallery. Both these divisions remain connected by a ciliated passage named as corridors, which are maintained in between the two lamellae (inter lamellar space) of a rosette. On the basis of above division Doving *et al.* (1977) demonstrated, that an unidirectional water current is created from vestibule to gallery via corridors. On the basis of the mechanism employed for the transportation of water through the olfactory chamber, the fishes can be divided into two groups : Isomates and Cyclosmates (Doving *et al.*, 1977; Doving and Thommesen, 1977). In the former group they placed carps, cat fish, eel and rocklings where only ciliary action creates water current through the olfactory chamber while in later group compression and expansion of accessory nasal sacs causes the water to pass through

the olfactory chamber. Doving *et al.* (1977) reported that in cyclosmates fishes the olfactory passage is not divided into vestibule and gallery.

In the present study contrary to the findings of Doving *et al.* (1977), *C. carpio* and *T. mossambica* bears insignificant vestibule and gallery and water is directly entered into the corridors through the anterior nasal opening. It was also found that *B. bagarius* possesses anterior and ventrolateral accessory nasal sacs respectively and can be placed in cyclosmates group. However, *B. bagarius* is also provided with well developed vestibule and gallery which is contrary to the findings of Doving *et al.* (1977). In *B. bagarius* the posterior lamellaeless part of the olfactory rosette contributes in the formation of well defined gallery

In the opinion of author, the division of olfactory passage on the basis of accessory nasal sacs seems to be an immature idea of Doving *et al.* (1977). The author is of the view that the division of olfactory passage depends upon the morphological structure of the olfactory chamber and the head. The elongated chamber will be having well defined vestibule and gallery while the short chamber will be devoid of such divisions. The division of olfactory passage is also seen distinctly well formed in the fishes where two apertures are situated at a considerable distance.

In *C. carpio* the nasal flap is dipped into the olfactory cavity, dividing it into anterior and posterior compartments. This ventral extension of nasal flap into the olfactory cavity is called as valance (Branson, 1963). The formation of valance is not reported in *B. bagarius* and *T. mossambica*. In the later fishes, the olfactory passage

has a continuous channel (the lumen) which on anterior side communicates to the anterior accessory nasal sac and posteriorly opens directly through the opening of the rosette. The corridors open into the lumen.

The Olfactory rosette :

The organ of olfaction are represented by a pair of olfactory sacs (chambers) which in sharks and rays are located on the central surface while in sturgeon and bony fishes on the dorsal surface of the head. The olfactory sac (chamber) is lined by the olfactory epithelium which is generally raised from the surface of the organ into complicated series or folds or lamellae to make a rosette (Hara, 1975). The shape of olfactory rosette varies greatly in different species. Bateson (1889) distinguished four types of lamellar arrangement : (i) in skate and dog fishes, the lamellae are arranged in radiating manner, line the septa of orange, (ii) in conger and eel, the lamellae are arranged in two rows on each side of the raphe, (iii) the lamellae are fitted together in a radiating manner forming a convex eminence in the olfactory chamber. It is either circular (*cottus*, *mottela mustela* etc.) or elliptical (*mackerel*, etc.). Such type of rosette is most common in fishes, (iv) the lamellae are arranged in single row generally parallel to the long axis of the body of the fish and the raphe is absent, eg. *Solea*, *Pleuronectus* etc.

Burne (1909) reported three types of the olfactory rosette : oval (in most of the fishes); round (in *ESOX*) and elongated (in *Anguilla*). Fishes with round rosette normally have only a few lamellae and usually show little response to the sense of olfaction. The species with oval rosette are most common, but, fishes with elongated rosette show

dominantly developed olfactory faculty. Teichmann (1954) tried to explain that the oval, circular and elongate rosette can be linked with his own first, second and third groups of eye-nose, eye and nose fishes respectively. In other words, Teichmann (1954) identified oval rosette with equally developed eye and nose faculties, circular rosette with predominantly developed optic faculty and elongated rosette with predominantly developed olfactory faculty.

In the present investigation it is found that the position of the olfactory chamber in the head and shape of the olfactory rosette vary greatly in the fishes selected for study. The olfactory rosette is oval in *C. carpio*, quadrangular in *T. mossambica* and elongated bean shaped in *B. bagarius*. The olfactory chamber is close to eye orbit in *C. carpio* and *T. mossambica* and close to snout in *B. bagarius*. Similar types of positions of olfactory chambers in different species of fishes have been reported in previous literatures by Branson (1963), Ojha and Kapoor (1971, 1972 a and 1973), Kapoor and Ojha (1972 and 1973), Sharma (1981), Yadav (1989), Dubey (1991), Chen and Arratia (1994) Byrd and Brunjes (1995), Sharma (2002) and Belanger *et al.* (2003). Therefore, the author is of the opinion that in carps it is generally situated close to the eye but in cat fishes it is close to the snout. In *T. mossambica* the rosette is quadrangular and is restricted close to eye due to the protrusible nature of jaws. Kapoor and Ojha (1971b) and Ojha and Kapoor (1973b) reported oval rosettes in *Garra gotyla* and *Glyptothorax telchitta* respectively and both have predominantly developed olfactory faculty. Bertmar (1972) suggested that both macrosmatic as well as microsmatic fishes may have oval rosette and thus shape has no concern with the efficiency of the olfactory organ.

In the present study it is found that *T. mossambica* with quadrangular rosette bears predominantly developed optic faculty but *C. carpio* with oval rosette have both faculties equally and predominantly developed. *B. bagarius* on the other hand show predominantly developed olfactory faculty and optic faculty is very much regressed. Thus, the author is of the opinion that oval or elongated rosette shows a great inclination towards the prominent development of the olfactory faculty although optic faculty may also be well developed. Therefore, on this basis the author concluded that macrosmatic fishes may be of two types : one having only olfactory faculty prominently developed (eg. *B. bagarius*) while other with both olfactory and optic faculties well developed (e.g. *C. carpio*). In microsmatic fishes, the olfactory faculty will be regressed but optic faculty will be well developed (e.g. *T. mossambica*). The author is of the view that the fishes with both the faculties well developed are more efficient in nature as compared to those having only one faculty predominantly developed and can be categorised into mesosmatic fishes.. The olfactory faculty in *T. mossambica* is morphologically giving an idea of its regression which suits to its predaceous habit as it visually targets the fish and other prey for engulfing them within its protrusible jaws. The olfactory rosette is supplemented with accessory nasal sacs which may bear the pressure of strong water current during protruding activity of jaws. *T. mossambica* can swallow fishes of half of its own size which can only be possible by visually objecting them.

In the present study except *T. mossambica* remaining two fishes (*C. carpio* and *B. bagarius*) are provided with anteroposterior

thickening, called raphe. The presence of raphe is very common in fishes and Burne (1909) observed raphe in 42 fishes out of 52 studied by him. Sheldon (1912), Teichmann (1954), Hansen and Zeiske (1993) also observed raphe in various fishes. Johnson and Brown (1962), Singh (1972), Bertmar (1972), Kapoor and Ojha (1973 a), Rahmani and Khan (1977, 1979), studied rapheless fishes and found that these fishes have comparatively lesser number of lamellae. Except Ojha and Kapoor (1973), Branson (1963) and Sharma (1981) nobody has discussed raphe histologically. Branson (1963) in *Hybopsis gelida* and *H. aestivalis* recalled it as central lamella containing ciliated and sensory cells but Ojha and Kapoor (1973) in *L. rohita* described it as nonsensory, nonciliated and secretory structure, allowing attachment to the other radial lamellae. Sharma (1981) reported that raphe in *E. denricus* is histologically identical to its lamellae but in *H. fossilis* and *N. notopterus* it differs in histological composition with their lamellae.

The author is of the opinion that raphe may be a modified lamella or it may be exclusively made up of the thickening of the floor of olfactory chamber. In both the conditions it permits attachment to the lamellae in different ways. It may be further concluded, that, fishes having elongated rosette may possess raphe as the thickening of the floor of olfactory chamber but in case of circular or oval rosette the raphe may be a modified lamella.

The author is also of the view that rapheless fishes possess regressed olfactory organ as compared to the fishes bearing raphe. Although some of the fishes having raphe also show microsmatic character, however, they are not as regressed as in rapheless fishes. Sharma (1981) reported microsmatic *E. denricus*, but it is not as

regressed as the fish devoid of raphe. Raphed fishes in majority exhibit macrosmatic character which indicates that raphe is an additional structure, increases the olfactory surface and permits attachment to the lamellae. It also facilitates proper reception of sensation through the water current.

The number, location, form and degree of development of folds (lamellae) in olfactory rosette of bony fishes vary significantly (Burne, 1909; Liermann, 1933; Schmal' hausen, 1962; Kapoor and ojha, 1972, 1973; Hara, 1975; Chen and Arratia, 1994; Fishelson, 1995). The largest number of lamellae was observed in the present study in *B. bagarius*, where olfactory rosette is provided upto 350 lamellae. The olfactory rosette of *Haplopagrus amentheri* have 230 lamellae, Barbot have more than 50 lamellae (Teichmann, 1954), Japanese eel have 90 lamellae (Shibuya, 1960), Bream have 34-36 lamellae (Bodrova, 1962), Pike and Salmon have 11-18 lamellae (Teichman, 1954 and Pfeiffer 1963), *Anguilla anguilla* have upto 70 lamellae (Teichmann, 1964), *H. fossilis* have 46-64 *N. notopterus* have 58-80. *E. denricus* have 11-16 and *M. armatus armatus* have 152-240 lamellae respectively (Sharma, 1981) and Oman shark have 28-32 lamellae (Fishelson and Baranes, 1997) In each case there is a successive increase in the number of lamellae as the size of the fish increases.

In the present study *C. carpio* bears 47-72, *B. bagarius* possess 91-128 and *T. mossambica* bears 14-19 lamellae respectively. In all these fishes lamellae show a trend of successive increase in their number with the growth of a fish.

Yamamoto and Ueda (1977, 1978 a,b,c,d,e) reported that the arrangement of lamellae in a rosette is either in two rows on each side

of the raphe or in a single row, arranged parallel to the long axis of the body or coming out from a single point. Kapoor and Ojha (1973) in *Channa punctatus* and Rahmani and Khan (1977) in *Anabas testudineus* reported the parallel arrangement of lamellae in a single row. Burne (1971, 1973a, b, 1974) and Kapoor and Ojha (1972, 1973), Sharma (1981), Yadav (1989) observed that most of the fishes bear two rows of lamellae on each side of the raphe in a rosette.

In the present study the most accepted arrangement of lamellae in two rows on either side of the raphe is seen in *C. carpio* and *B. bagarius* but in *T. mossambica* the arrangement of lamellae is of peculiar type, roughly in the form of lotus petals. In *T. mossambica* quadrangular rosette is befitted in the olfactory chamber but bears lamellae on its anterior side, leaving posterior part lamellaeless. The lamellae in all the forms of present study are so arranged that inter lamellar space are maintained in between the two lamellae and permit the water circulation through them.

The author observed that the lamellae show a clear cut increase in their number with total length of all the fishes in the present investigation and it is in agreement with the findings of Bateson (1889), Burne (1909), Liermann (1933), Teichman (1954), Eaton (1956), Johnson and Brown (1962) Schmal' hausen (1962), Pfeiffer (1963, 1964), Kleerekoper (1969), Ojha and Kapoor (1971, 1972, 1973 a, b, 1974), Kapoor and Ojha (1972a, 1973a, b), Hara (1975), Doving and Thomesen (1977), Sharma (1981), Yadav (1989), Hansen and Zeiske (1993) and Sharma (2002). However Pfeiffer (1962) reported in *Onchorhyncus* that the number of transverse lamellae increases with the growth of the fish upto a certain extent and then remains

relatively constant. Rahmani and Khan (1977) in *Anabas testudineus* found that in adult fish the number of lamellae varies from 7 to 10 and the correlation can be established between the number of lamellae and size of the fish. Devitsyna (1972) is of the opinion that the number of lamellae remains relatively constant and is a characteristic of each species. Consequently, the enlargement of receptor surface is at the expense of an increase in area of the lamellae and not in their number.

Burne (1909) reported that considerable differences are apparent in the shape of individual lamellae of the rosette. Starting from the type present in *Gadus* as centre (Rosette, Column V), one line of variation leads by the suppression of the periphery part of the lamellae and the exaggeration of the linguiform process (Rosette, Column-VI) to a claw like shape which is peculiar characteristic of the rosette of the salmonidae and clupeidae.

In the present study the lamellae of *C. carpio* and *B. bagarius* bear linguiform process in the middle of distal region of their dorsal surface. The shape of the lamellae in *C. carpio* and *B. bagarius* is greatly affected with the presence of the linguiform processes and they are leaf and thumb-shaped respectively. In *T. mossambica* the lamellae directly emerge out from the floor of olfactory surface and thus exhibit no trend of suppression and exaggeration. The linguiform process is also termed as "thumb" by Doving *et al.* (1977) and it divides the olfactory chamber into central and peripheral chambers. In *C. carpio* and *B. bagarius* the linguiform process divides the central cavity of olfactory chamber into central and peripheral channels but in

T. mossambica there is no such formation of central cavity of olfactory chamber because it is rapheless and devoid of linguiform process.

Histological findings reveal that the olfactory lamellae of *C. carpio* show a tendency of bifurcation and trifurcation. Here crypt formation are also reported which accommodate large number of receptor cells and take a shape of olfactory crypt. In *B. bagarius* minor and curved lamellae are also observed in the present study. In *B. bagarius* posterior lamellae are seen forming the bud which after detachment from mother lamellae attach to the adjacent recipient lamellae, adding a rapid growth of the recipient lamellae. The curving of the terminal end of some lamellae has been observed in *B. bagarius*. Bertmar (1972), Kapoor and Ojha (1973, 1974), Rahmani and Khan (1980) and Sharma (1981) reported the extrusion of cells from the terminal end of the lamellae but in present study the lamellae of *C. carpio* and *B. bagarius* show a tendency of discharging the "Cell ball" from their terminal ends. In *T. mossambica* different types of crypts and other microformations like filiform, fungiform, cuneiform etc. are observed in the middle and hinder lamellae. All these observations contribute to the new findings of the present investigation and may significantly be linked with the morphogenetic activity of the olfactory epithelium.

In *C. carpio* the formation of crypts of different shapes and sizes and other microformations in the receptive olfactory surface is due to the tremendous muciferous activity of goblet cells. This sequential adaptive modifiactions in the olfactory epithelium of *C. carpio* is due to the result of acclimatization process from its original Black and Caspean sea of Turkistan to fresh water conditions of plains and

altitude regions. Under its changed habitat it is giving good cultivable results with major carps and other exotic fishes in different parts of India.

In *B. bagarius* there occurs tremendous elongation of posterior lamellae to accomodate them in wide olfactory chamber space. The middle lamellae also shows elongation of the lamella but not to the extent shown by posterior lamellae. This elongation in the lamellae is due to the active mitotic division of the basal cells. The author has also observed the curvings at the distal end of the posterior lamella due to the elongation of the lamellae in *B. bagarius*. In *B. bagarius* the posterior lamellae are also seen forming lateral bud which after detachment from mother lamellae attach to the recipient lamellae and thus enhancing the rapid growth of recipient lamellae. The posterior lamellae are also shown discharging "cell ball" from their terminal ends. The author has also observed the formation of basal minor lamella from the mother lamella by the process of outpushing of submucosa and mucosa of the later. The minor lamella later on becomes an independent lamella. The author is of the opinion that these morphogenetic movements results in the increase of olfactory receptive surface in *B. bagarius*.

In *T. mossambica* different types of crypts and other microformations like filiform, fungiform, cuneiform etc. are observed in the middle and hinder lamellae. The author is of the opinion that these different types of microformations results due to the infolding of lamella. This infolding in the lamella occurs due to the activity of basal cells and also to accomodate these lamellae in the smaller olfactory chamber space.

Ecological Co-efficient :

The ecological coefficient can be calculated by the relative lengths of the telencephalon and mesencephalon and by the areas of the olfactory rosettes and the retinae. It gives distinctive results which can illustrate microsmatic, macrosmatic and mesosmatic nature of the fishes under study. In the present investigation, area of the olfactory surface and of the eye and lengths of the telencephalon and mesencephalon are taken as parameters to calculate the ecological coefficient of the fishes under study.

According to Rahmani and Khan (1981) it is easy to calculate the area of the eye, but the olfactory surface represents difficulties because in some fishes villi like secondary foldings are developed which increases the olfactory area. In Teichmann's method (1954) which is adopted here, no consideration is given to these secondary lamellae. Moreover, it is difficult to calculate the area of these secondary foldings without destroying the lamella. The secondary folds are not uniformly developed, so, no method could be adopted to calculate the area. Thus in those fishes which have secondary foldings, the areas of the olfactory surface would be higher than calculated by usual methods.

In the present study the author has also observed different types of microformations in the olfactory epithelium of fishes under study. The area of these microformations cannot be calculated by usual method. Therefore, the area calculated by the usual methods excludes these microformations, and cannot be considered as accurate measurement. But the area calculated by usual methods gives sufficiently good evidence for discriminating the fishes under

study as macrosmatic, mesosmatic and microsmatic fishes. In order to overcome these drawbacks the author also calculated the ecological coefficient by the length of the lobes of the brain.

Taking into consideration the above drawbacks in each method, the present investigator has compared the two results and then concluded about the habit of the fish. The present study reveal that there are more chances of error by adopting only Teichmann's method (1954). Therefore, both the brain-lobe method and olfactory area method should be adopted and then conclusion should be drawn regarding the habit of the fishes understudy.

In the case of *C. carpio*, though the area of two rosettes is higher but the value of the area of two retinae cannot also be ignored. The significant valuation of both the faculties (olfactory and optic) as well as slightly higher length of mesencephalon than telencephalon, indicate mesosmatic nature of above mentioned fish, where both the faculties play their significant role in the habit of the fish. This conclusion is in agreement with the highly active *C. carpio* which swims near the surface and feeds voraciously by rapid protruding and retracting the jaws. It easily becomes pet to the master due to its highly sensitive optic and olfactory faculties.

In *B. bagarius* the area of two rosettes and length of telencephalon is considerably higher than the areas of two retinae and length of mesencephalon, which discriminate it as macrosmatic fish. This conclusion is in agreement with the natural habit of the fish which is bottom dwelling, living in mud-holes and hunting in dark. These characters are in accordance with feeble developed optic faculty.

and thus olfactory faculty significantly aid in the livelihood of *B. bagarius*.

T. mossambica gives an idea of its microsmatic nature, as the area of two retinae and those of mesencephalon is higher than those of two rosette and telencephalon. *T. mossambica* shows predominantly developed optic faculty which can be correlated with its highly predaceous nature, preying on small cyprinids and visually dash out to capture them. Hence, *T. mossambica* is a microsmatic fish and depend on optic faculty in discharging the activities required for the successful existence of this fish.

The author is of the opinion that irrespective of macrosmatic, mesosmatic and microsmatic nature of the fishes under study, the function of the optic faculty cannot be ignored though its degree of efficiency varies with respect to olfactory sensitivity. Except for the fishes of abyssopelagic zone of the sea, dark caves and very turbid waters, where vision is minimum or nil, most of the fishes utilize both vision and olfaction for day to day behaviour. In eye-nose and even in nose fishes, vision has an important role to play. The olfactory organ of fishes has a low threshold (Teichmann, 1959; Kleerekoper, 1969; Hara, 1975). The food source or a companion gives off its odour which diffuses and diminished with distance in accordance with something like inverse law of gas diffusion. The concentration of the odour falls off rapidly with distance from the producer. When the receiver receives the odour, it becomes excited and follows the odour gradient. If the receiver is very far off from where the gradient has levelled, the excitement and increased activity of the receiver might bring it nearer to the source where the gradient may be useable. Once the receiver is

near the source of the odour, its vision now becomes more important. Thus, though a fish may be macrosmat, mesosmat and microosmat, both vision and olfaction complement each other and plays an important role in its behaviour.

Route of water circulation:

In all the three fishes under investigation, namely, *C. carpio*, *B. bagarius* and *T. mossambica*, it is observed that the water enters into the olfactory chamber through anterior nasal opening and leaves out through the posterior nasal opening. In other words it can be said that unidirectional flow of water exists in these fishes.

Doving *et al.* (1977) reported that the direction of ciliary beat is consistent with the direction of the water current i.e. the cilia beat from anterior to posterior side of the olfactory chamber. This beating of cilia constantly creates a water current from anterior to posterior direction. All the three fishes selected for the present study possess cilia in their olfactory epithelium which help the water to circulate in the antero-posterior direction of the olfactory rosette.

In addition to antero-posterior beating of cilia, the anterior nasal opening is always situated either on a tube or thickened lip or thickened border but posterior nasal opening is wide and flush with general surface of the head of the fish. This architectural pattern indicates that in forward movement of fish, water will compulsory enter through anterior and exits through posterior nasal opening after irrigating the olfactory epithelium properly. The posterior nasal openings of *B. bagarius* and *T. mossambica* are wide and valved, which helps in the proper functioning of accessory nasal sacs present in these fishes. In case of *C. carpio* the posterior nasal opening is

considerably wide and is without valve, allowing a constant contact of water with the olfactory epithelium in either moving or in stationary state.

According to Doving *et al.* (1977) when the movement of water across the olfactory chamber is brought about by ciliary action, the fishes are called as isosmates and when it involves the compression and expansion of the accessory sacs, the fishes are placed under cyclosmate category. In the present study *C. carpio* comes under isosmate category while *B. bagarius* and *T. mossambica* comes under cyclosmate category. In *C. carpio* and *T. mossambica* the circulation of water takes a shortest route due to close location of anterior and posterior nasal openings. In *B. bagarius* the route of circulation of water through the olfactory chamber is considerably longer as their nasal openings lie at a distance extending from the snout to the eye orbit.

Accessory nasal sacs :

In the present study, the detailed histological aspect of accessory nasal sac is described in *B. bagarius* and *T. mossambica*. In *B. bagarius* the sac is named as ventrolateral accessory nasal sac, as it is situated ventrolaterally to the olfactory chamber in association with olfactory rosette. In *Tilapia mossambica* a well developed ethmoidal and lacrymal accessory nasal sacs is present in relation to ethmoid and lacrymal bones. These sacs open by their separate openings underneath the vulvular posterior nasal opening. In *T. mossambica* the ethmoidal sac are long, mediodorsal and lies slightly above the main olfactory chamber, while the lacrymal sac are small, narrow and extends to the length of maxillaries. In *B. bagarius* the

ventrolateral accessory nasal sac is an extension of the olfactory epithelium to the ventrolateral side of the olfactory chamber. In *B. bagarius* the ventrolateral accessory nasal sac is composed of cuboidal supporting cells, basal cells and goblet cells. The excessive mucous secretion through these sacs in present fishes lubricates total olfactory passage protecting it from mechanical harms and entangling unwanted foreign bodies into it which latter on flushed out with outgoing water current. Mucous secretion also help in olfactory reception by holding reactionary content near the receptive surface.

The accessory nasal sac in both the fishes under investigation are constituted of outer mucosa and inner submucosa. It is perfectly non-sensitive secretory structure, encircling a lumen for the accumulation of water. The mucosa is found to be composed of cuboidal supporting cells, basal cells and beaked or simple mucous secretory goblet cells. Receptors are totally wanting in the accessory nasal sacs of both the fishes under investigation. The submucosa is made up of connective tissue fibres, fibroblasts, histocytes, lymphocytes, basal cells, blastema cells and pigment cells. Rahmani and Khan (1981), Sharma (1981), Dubey (1991), Sharma (2002) and Belanger *et al* (2003) reported accessory nasal sacs revealing same histological concept as reported in *B. bagarius* and *T. mossambica*.

The findings highlighted by Rahmani and Khan (1981) Sharma (1981) and Belanger *et al* (2003) are restricted to the concept that they are additional accommodation for water in the olfactory chamber. However, the present findings has revealed that they are not only pumping structure for forcibly drawing the water current through the olfactory surface for convenient and prompt reception of olfactory

chamber but also to lubricate the whole olfactory passage, protecting it from mechanical harms by forceful circulation of water current. The presence of different type of accessory nasal sac in the olfactory chamber of *B. bagarius* and *T. mossambica*, serve as histoeological supporting device to these fishes and help them to withstand successfully in their prevailing environment.

Discussion on Histology :

The olfactory epithelium of vertebrates is known to comprise of olfactory receptor cells, intermingled with supporting cells (Hopkins, 1926; Kolmer, 1927; Allison, 1953; Bloom, 1954; Le Gros clark, 1957; De Lorenzo, 1957; Ottoson, 1963; Porter and Bonneville, 1964; Frisch, 1967; Moulton and Beidler, 1967). Other cellular components are basal cells and mucous secretory goblet cells. The fine structure of the olfactory epithelium has been studied in number of species of fishes by Trujillo-Cenoz (1961), Bannister (1965), Bronshtein and Ivanov (1965), Vinikov (1966), Wilson and Westerman (1967), Thornhill (1967), Gemne and Doving (1969), Kleerekoper (1969), Schulte and Holl (1971), Bertmar (1972a) Ojha and Kapoor (1973), Kapoor and Ojha (1974), Hara (1975), Yamamoto and Ueda (1977, 1978 a-f), Zeiske *et al.* (1979) Theisen *et al.* (1980), Rahmani and Khan (1980), Sharma (1981), Yadav (1989), Dubey (1991),. Hansen and Zeiske (1993), Byrd and Brunjes (1995), Fishelson (1995) and Eastman and Lannoo (2001, 2003, 2004).

It is commonly observed that the basic plan of olfactory epithelium of fish shows no fundamental variation from the general vertebrate pattern. Histologically the lamella of all fishes consists of two principal layers, an outer sensory epithelium or mucosa and a

central core or submucosa. The relative thickness of mucosa and submucosa varies from fish to fish and some times even in the lamellae of a rosette. The basement membrane stands as partition in between mucosa and submucosa and serves as medium for the exchange of nervous and nutritional components.

In the present comparative study similar cellular organization of the olfactory epithelium, with individual variation in the arrangement and shape of a particular cell type has been observed in *C. carpio*, *B. bagarius* and *T. mossambica*. It is also observed that the three fishes under investigation which belong to different habitat, exhibit different levels of morphogenetic activity in the olfactory epithelium which results in the formation of bulging, hillock elevation, depression, ciliary clustering, narrowing, swelling, curving, budding and cell balls of various shapes and sizes. These morphogenetic activities is due to the migration of basal cells through the cellular spaces created by the fusion or death of goblet cells after discharging their mucous content.

Supporting cell :

Hopkins (1926), Kolmer (1927), Allison (1953), Branson (1963), Watling and Hilmann (1964), Bannister (1965) Ojha and Kapoor (1974) Moran *et al.* (1992), Byrd and Brunjes (1995) and Fishelson (1995) reported the presence of only ciliated supporting cells in the olfactory epithelium of fishes. Holl (1965), Bertmar (1972), Rahmani and Khan (1980) Sharma (1981), Yadav (1989), Dubey (1991) described the presence of ciliated and non ciliated supporting cells in the olfactory epithelium of some teleost species. According to Ojha and Kapoor (1973) each supporting cell in *Labeo rohita* bears 8-12 cilia implanted on basal bodies.

In the present study the ciliated and nonciliated supporting cells in *C. carpio* are intermingled in the proximal region of each lamella but in distal and middle regions they are exclusively ciliated. In *T. mossambica* ciliated supporting cells are present in uniform olfactory epithelium but in the its transitional condition they are nonciliated and have short body. In *B. bagarius* the author has traced the presence of ciliated, non ciliated and transitional supporting cells arranged in well demarcated zones. In this fish the distal zone is nonciliated while proximal and middle zone are exclusively ciliated. Bertmar (1972a) reported that there is no difference between the two types of supporting cells in their abundance or relation to the receptors but when the supporting cells lie together, the groups consists of one type of cell. In accordance with Bertmar's view and the findings of Yadav (1989), Dubey (1991), Singh (1992) and Sharma (2002), the grouping of ciliated and nonciliated supporting cells reaches upto such extent in *B. bagarius* that the ciliated and nonciliated zones are clearly visible.

In *C. carpio* the supporting cells are subjected to a process of continuous transformation into the mucous secretory goblet cells, therefore, whole of the peripheral or distal surface of the lamellae is seen occupied by the theca of goblet cells. The supporting cell in *C. carpio* can be seen in proximal region of the lamellae but in middle and distal regions they are rarely observed in original form. The transitional stages of supporting cells can be frequently seen in the middle and distal region of the lamellae of *C. carpio*. Ojha and Kapoor (1973) in *L. rohita* and Kapoor and Ojha (1974) in *Channa punctatus* observed the supporting cells transforming into the goblet cells. But in

C. carpio the transformation of supporting cells in mucous secretory goblet cells is on mass level and has yet not been noticed in any fish studied so far. The proximal intervening region of the lamellae of *C. carpio* show that the supporting cells may secrete mucous before their transformation into goblet cells. This is in accordance with Kapoor and Ojha (1974) that apart from their supporting function, the supporting cells in the olfactory epithelium have been assigned a role of secretion and isolation of receptor cells. On the other hand Gerebtzoff and Shekapenko (1952) and Gerebtzoff (1953) contradict the idea regarding the secretory nature of supporting cells. Histological observations reveal that the ciliated supporting cells of *C. carpio* are supplied with muciferous cytoplasm which may convert in the secretory fluid (mucous) and hence, the supporting cells is transformed into a goblet cell.

In *T. mossambica* the supporting cells are subjected to great displacement due to tremendous flow of basal cells to any peripheral direction of the olfactory mucosa. This leads the peripheral or distal surface of lamella to convert in peculiar form of elevations or deepenings. The former may be in the shape of cuneiform, filiform, fungiform, hillock and minor elevations whereas the later in the shape of tubule, flask, funnel and vacuole like deepenings. The flow of basal cells makes the olfactory epithelium transitional which is supposed to give rise to any of the form described above for the purpose of enhancing the olfactory area, as well as to accommodate the flow of basal cells. The transitional stage of supporting cells is also observed in *C. carpio* where muciferous nature of olfactory epithelium followed

by the flow of basal cells results in specific formations on the lamellar surface.

In *B. bagarius* the ciliated supporting cells are confined to the proximal and middle region of the initial and middle lamellae on both sides of the raphe, demarcating supporting and sensory zone, while the distal zone is provided with nonciliated columnar supporting cells intermingled with mucous secretory goblet cells. Thus there occurs a clear cut demarcation between negligible secretory ciliated and non ciliated highly secretory zone. The author shares the view of Rahmani and Khan (1980), Sharma (1981), Yadav (1989), Dubey (1991), Singh (1992) and Sharma (2002) that the grouping of different types of supporting cells in a zone or in small groups may be for some functional significance as reported in *B. bagarius*. Histological observations reveal that some of the nonciliated supporting cells of *B. bagarius* are supplied with muciferous cytoplasm which may be transformed into goblet cells. Such cells are denominated as transitionary supporting cells. The degree of occurrence of goblet cells depends upon the habit and habitat of fish.

In the present study the author observed cilia in all the three fishes (*C. carpio*, *T. mossambica* and *B. bagarius*) belonging to different habit and habitat, but Gooding (1963) in *Katsuwonus pelamis* and Gemne and Doving (1969) in *Lota lota* observed total absence of cilia in the supporting cells. Bannister (1965) has also not described cilia in *Phoxinus phoxinus* and *Gasterosteus* but reported that free surface of these cells bear number of irregular microvilli. In *B. bagarius* and *C. carpio* the supporting cells are uniformly ciliated but at the periphery of the lamellae the ciliation becomes more prominent forming tuft of

cilia, which helps in creating water current. In *T. mossambica* clustering of cilia is not observed and single cilium is coming out from the outer limb of the supporting cell. Thus, it can be inferred at this juncture, that the degree of ciliation depends upon the amount of work loaded on supporting cells. Therefore, in case of *B. bagarius*, water has to travel long distance in olfactory chamber and has to bath enormously developed olfactory surface, therefore, ciliation is predominantly developed and that too in clusters. On the contrary, the olfactory epithelium of *T. mossambica* bears light ciliation because in them the well developed ethmoidal and lacrymal accessory nasal sacs operate the water circulation through the olfactory chamber. Thus, the author is of the opinion that the fishes which are totally dependent on ciliary mode of water circulation through the olfactory chamber are provided with clusters of cilia and exhibit more muciferous activity in the supporting zone. Although *C. carpio* is a eye-nose fish but its olfactory faculty is predominantly supporting to its habit and habitat. The tremendous ciliation in olfactory epithelium advocates its tendency of dependence on this particular device for substituting prompt and efficient involvement of water current with its surface.

Holl (1965) suggested that the nonciliated supporting cells isolate the receptors and contribute to the metabolism between olfactory epithelium and blood, whereas, ciliated types act for the distribution of mucous on the epithelial surface. Holl (1965) and Pipping (1926) were of the view that ciliary activity of olfactory epithelium creates water current through the olfactory chamber. De Lorenzo (1960) pointed out that supporting cells may be involved in

the perception of the sense of olfaction in some way or other. But Kapoor and Ojha (1974) treated this view as an erroneous conception. The author is also of the view that marked and frequent existence of the receptor cells in the olfactory epithelium of fishes under study, leave no chance for supporting cell to perform the function of reception. Therefore, the supporting cells are meant for the maintenance of integrity of the olfactory epithelium and to provide mechanical support to the dendrite of the receptor cells, keeping them erected in the position for the reception of senses from the water current passing through the olfactory chamber. Thus, inference can be drawn from foregoing description that ciliation is an integral part of supporting and receptor cells. The cilia of supporting cells are essential for drawing water current, while the cilia of receptors act as antennae for reception of olfactory sensation from the circulating water. However, the ciliation in supporting cells may disappear in some places where these cells are subjected to muciferous activity of underneath pressure of flowing basal cells. The other type of cellular projections like microvilli etc. are not of supporting cells but are the projection of receptor cells.

In the present study, transitional supporting cells have also been noticed in all the three fishes under investigation. Their major concentration is reported in the region of the olfactory mucosa which are either under the pressure of flow of basal cells in specific microformation or in the regions through which lamellar emergence is anticipated. The presence of transitional supporting cells were also reported in the findings of Ojha and Kapoor (1973), Kapoor and Ojha (1974), Rahmani and Khan (1980), Sharma (1981), Yadav (1989),

Dubey (1991), Singh (1992) and Sharma (2002). Bertmar (1972) and Hara (1975) has denied the existance of transitionary supporting cells.

Receptor cells :

The sensory epithelium is characteristically provided with the elements which perceive sensation through the medium in which a particular organism exists. The sensory elements can be identified in the form of receptor cells, which have got a long history of their investigation in the olfactory epithelium of teleosts by Aichel (1895) in *Salvelinus alpinus*, *Coregonus worthmanni* and *Salmo trutis*. Singh (1972), Rahmani and Khan(1980), Sharma (1981), Yadav (1989) and Sharma (2002) reported that receptor cells are almost uniformly scattered in young fish but in adult fish they are grouped and burried in depression in between the secondary lamellae. Malykina *et al.* (1962), Gemne and Doving (1969), Yamamoto and Ueda (1977), Muller and Marc (1984) and Zielinski and Hara (1998) on the contrary reported that the sensory elements are not uniformly distributed among the other cellular components. Doroshenko and Motankin (1987) explored the variability in the distribution of cellular elements to the extent that the olfactory epithelium is distinct into receptory and indifferent epithelia.

In the present investigation, author observed that the distribution of receptor cells varies greatly in all the three fishes depending upon the nature of the olfactory epithelium. In *C. carpio*, three types of receptor cells are found, namely; primary neurons, rod shaped and spindle shaped receptor cells. In *B. bagarius* the receptor cells observed are primary neurons and spindle shaped cells while in *T. mossambica* primary neurons and rodshaped receptor cells are

found. It is also observed that the distribution and concentration of these receptor cells varies in these fishes. In *C. carpio* the receptor cells are irregularly distributed while in *B. bagarius* they are regularly distributed. In both these fishes the concentration of receptor cell can be seen in crypts present at different depths of the olfactory epithelium. In *C. carpio* these cells are commonly found in the empty theca of marginal goblet cells, among the ciliated and non ciliated supporting cells and in between the theca of marginal goblet cells. In *T. mossambica* the receptor cells are irregularly distributed in all the places of olfactory epithelium and they are concentrated in specific deepening of crypts which are created by the regular mitotic division of basal cells and subsequently to accommodate this enormous bulk, the olfactory epithelium gets infolded, forming exposed and unexposed crypt like deepening. The concentration of receptors at one place can be observed in these crypts which are in the form of flask, funnel, tubule and vacuole like. The rod shaped receptors and may be rarely present in the adjacent elevations in the shape of cuneiform, filiform and fungiform.

In *C. carpio* the rod shaped receptor cells are confined in the supporting zone having considerably thick dendrite and enormously elongated axon. They end terminally by an expanded tip which bear microvilli. In *B. bagarius* the rod shaped receptor cells are commonly found in middle and distal region. The dendrites of these cells are thick and found extending in between the theca of two goblet cells or transversing singly or in groups through the empty theca. In *T. mossambica* the rod shaped receptor cells are observed on the general surface of the olfactory epithelium where basal cells have not shown

their migratory activity. The presence of rod shaped receptor cells in these fishes in an accordance with the findings of Bertmar (1972) who reported these receptor types in sea trout. The author is of the opinion that the presence of rod shaped receptor cells indicate high sensitivity of these fishes with respect to the olfactory behaviour because in higher vertebrates rod shaped receptor cells are common in occurrence (Kolmer, 1927 and Neuhaus, 1955).

Dogiel (1887), Morril (1898), Jagodowski (1901) and Castello (1956) reported spindle, conical and columnar receptor cells in fishes and frogs. In *Salmo*, rod and spindle shaped receptor cells are described by Holl (1965). He further reported spindle shaped receptor cells in all teleosts studied by him whereas the rod shaped receptor cells was only found in *Salmo goirdneri*, *Salmo trutta fario*, *Esox lucinus*, *Pleuronectus platessa* and *Trigla corex*. In present study the spindle shaped receptor cells in *C. carpio* lie in the middle of mucosa, sending their very much elongated dendrite and axon to distal and proximal regions respectively. In *T. mossambica* the spindle shaped receptor cells are absent. In *B. bagarius* these cells corresponds to secondary neurons and are found alongwith abundant primary neurons and supporting cells in crypts and deepenings. In this fish the deepenings are supplied with spindle shaped receptors. The dendrites of these cells in *B. bagarius* establish synaptic contact with the axon of primary neurons. The synapse formation is an indication in the enhancement of olfactory reception capacity and may be described as advanced nervous character reported in the olfactory mucosa of *B. bagarius*. This finding is in accordance to the findings of Ojha and Kapoor (1973) in *Labeo rohita* and Kapoor and Ojha in

Channa punctatus. They reported that secondary neuron or spindle shaped receptor cells establish synaptic contact with primary neurons before reaching upto the mitral cells of the olfactory bulb. On the other hand there is no synaptic contact between any two receptor types in *C. carpio* and *T. mossambica*, which is in accordance with the findings of Ottoson (1963), Yamamota *et al.* (1965), Moulton and Beidler (1967), Kleerekoper (1969), Graziadei and Metcalf (1971), Rahmani and Khan (1980) and Sharma (1981). They described that similar to other vertebrates, fishes are characterised of making direct synaptic contact with the mitral cells of the olfactory bulb and not in the olfactory epithelium.

The primary neurons in *C. carpio* and *T. mossambica* are richly concentrated in the specific deepenings, taking a shape of olfactory crypt. In such formations, the dendrites are concentrated, sending their short and elongated stereocilia into the interlamellar spaces for the reception of olfactory sensation through the circulating water current. The formation of olfactory crypt takes place in the spaces created by the empty theca of goblet cells. This finding by the present author is a new one because no where such peculiar formations are reported in the fishes studied so far. In case of *B. bagarius* the primary neurons are abundant alongwith spindle shaped receptors and supporting cells in crypts and deepenings, but are distributed uniformly at the periphery. The dendrite of spindle shaped receptor cells establish synaptic contact with the axon of primary neurons in *B. bagarius*.

The distal tips of the dendrites of the receptor cells in fish and cyclostomes swells into an olfactory vesicle which bear olfactory hair

(cilia) (Jagadowski, 1901, Vinnikov, 1956; Trujillo-Cenoz, 1961; Bronshtein, 1963, 1965; Bannister, 1965; Kleerekoper, 1969; Ojha and Kapoor, 1973; Kapoor and Ojha, 1974; Rahmani and Khan, 1980; Sharma, 1981; Singh, 1982; Muller and Marc, 1984; Yadav, 1989, Dubey, 1991; Singh, 1992; Zielinski and Hara, 1998; and Sharma, 2002). In *C. carpio* and *T. mossambica* the rod shaped receptor cells are microvillous, implanted on their terminal ends, but the long dendrite of spindle shaped receptor cell in *C. carpio* end terminally in the form of olfactory vesicle with elongated cilia. On the contrary, olfactory vesicle formation in *B. bagarius* has not been observed in the present study and terminal end of dendrites as such project in the form of cilia of different sizes.

Wilson and Westerman (1967) reported cilia and microvilli on the same receptor cells in *Carassius auratus*, similar to the findings of Malykina et al. (1969). The olfactory receptor cells of *C. carpio*, *B. bagarius* and *T. mossambica* differ in size, number and structure of the olfactory cilia and vesicle which probably reflect the functional heterogeneity of the sensory cells.

Yamamoto and Ueda (1977) identified four types of receptor cells on the basis of the surface specialization : (i) the first type bears 10-30 relatively long cilia on a wide and flat surface. All the cilia of this type are inclined in the same direction over the wide range of epithelium. This is called type one ciliated cell (latter they added that the cilia of type one ciliated cells may be motile and it might be associated with the circulation of fluid between the lamellae). (ii) type two ciliated cells has 8 to 12 short cilia which project radially from the round apex of the cell, (iii) the third type has a tuft of hundred or more

microvilli cells (iv) the fourth is rod cell which neither bears cilia nor microvilli and its apical end produce in the form of a simple rod from the epithelial surface. Bannister (1965) and Schulte (1972) also described type (i) ciliated cells but they regarded these cells to be nonsensory. Ichikawa and Ueda (1977) by the use of retrograde technique reported that type (ii) ciliated cells and microvillous cells are genuine receptor cells, because when olfactory nerve is transected only these two types of cells degenerate while the type (i) ciliated cells and rod cells remain unaffected. This proves that type (i) ciliated cells are not receptor cells.

In the present study type two ciliated cells corresponds to the receptor cells of *B. bagarius* where cilia are comparatively short and less in number projecting into the interlamellar spaces. The rod shaped receptor cells of *C. carpio* and *T. mossambica* corresponds to type three ciliated cells where microvilli are observed. But the protruding end of primary neurons in the crypts or empty theca in *C. carpio* and *T. mossambica* may be identified as type four receptor cells as described by Yamamoto and Ueda (1977).

Mucous Secretory Goblet Cells :

The mucous secretory goblet cells are important cellular components of the olfactory epithelium of fishes and are found distributed among the supporting cells. Kubiak (1962), Bannister (1965), Pfeiffer (1963), Kleerekoper (1969), Bertmar (1972), Singh (1972), Devitsyna (1972), Ojha and Kapoor (1973), Kapoor and Ojha (1974), Sharma (1981), Yadav (1989), Dubey (1991), Singh (1992), Getchell and Getchell (1992), Sharma (2002) described the presence of goblet cells in different species of fishes.

In the present study goblet cells and its mucous secretory activity is prominently noticed in the olfactory epithelium of *C. carpio* and *B. bagarius* but in *T. mossambica* such activity is very restricted and the presence of goblet cells is rarely noticed in olfactory mucosa but there activity in accessory nasal sac is dominantly noticed.

The absence of goblet cells are also reported in *Xenentodon cancila* (Singh, 1972), *Hybopsis gelida* and *H. aestivalis* (Branson, 1963), *Colisa faciatus* (Rahmani, 1979), *Anabas testudineus* (Rahmani and Khan 1980) and *N. notopterus* (Sharma, 1981). Holl (1965) described mucous cells in both indifferent and sensory epithelium of salmo, especially in those places where secondary foldings occurred. Bertmar (1972) also found in salmo that mature goblet cells lie in surface zone, especially of the indifferent epithelium, but also lie scattered in sensory epithelium. The presence of mucous secretory goblet cells are also observed in the deeper zones of mucosa in *Labeo rohita* (Ojha and Kapoor, 1973) and *Channa punctuatus* (Kapoor and Ojha, 1974).

In higher vertebrates the olfactory epithelium is kept moist by the secretion of Bowman's gland (Allison, 1953). This gland is absent in fishes, however, in place of it unicellular goblet cells compensate the function of Bowman's gland (Getchell and Getchell, 1992).

In air breathing vertebrates, the supra-epithelial mucous layer dissolves the particles to be smelled and wash away the material that has already been detected, so that sample of air can be examined (Hildebrand, 1974). In fishes there is no need for the dissolution of the material to be detected because it is already in liquid form and the constant flow of water washes away the material that has been

detected. Therefore, the presence of large number of mucous cells in the olfactory epithelium of fishes could be explained by the fact that the secreted mucous forms a boundary for the water flow in the olfactory epithelium (Zeiske *et al.* 1976). The statement of Andres (1966) that in fishes the free surface of receptor cell is directly rinsed by the water flow is not correct (Zeiske *et al.* 1976). The mucous secretion is overlapped on the olfactory surface and thus probably helps in smooth flow of water in the olfactory chamber. Rosen and Cornford (1971) reported that the slime has a remarkable capacity to decrease greatly the friction of water in the pacific barracunda, *Sphyrna argentea*, for example, the friction of water decreases by as much 65.6%.

The olfactory epithelium of *C. carpio* is abundantly supplied by the mucous secretory goblet cells which are seen at different stages of their formation at variable depths. Among supporting cells, they are found to be uniformly arranged in the form of marginal goblet cells.

In *B. bagarius* the mucous secretory goblet cells are confined in the distal region of the initial and middle lamellae but can be encountered anywhere in hinder ones. The proximal and middle regions of the initial and middle lamellae are devoid of goblet cells. In *T. mossambica* the olfactory mucosa is scantily supplied with goblet cells but they are richly present in accessory nasal sacs.

Bloom and Fawcett (1978) quoted that in mammals the only unicellular glands are the mucous secretory goblet cells which lie scattered among the columnar cells of the epithelium on many mucous membranes. They further pointed out that goblet cells secrete mucous and are made up of an expanded apical end, filled with pale

droplet of mucin. The basal end contains compressed nucleus and a small amount of deeply staining cytoplasm. The expanded cup shaped structure is known as theca which remain associated with the basal zone by a thin base like stem.

The structure of goblet cells in the olfactory epithelium of *C. carpio* and *B. bagarius* is in accordance with the structure described by Bloom and Fawcett (1978) with reference to mammals. In *C. carpio* and *B. bagarius* the theca is expanded cup shaped with clearly visible nuclear complex which takes a shape of compressed structure. In *T. mossambica* the goblet cells are rounded, giving inconspicuous visibility of theca and its other associated structures.

Ojha and Kapoor (1973), Kapoor and Ojha (1974), Sharma (1981) Doroshenko and Motavkin (1987) and Yadav (1989) described varying shapes and sizes of the goblet cells with different phases of their secretory activity. In the present study the shape of the goblet cells in *C. carpio* and *B. bagarius* show great variations. In these two species goblet cells are mostly beaked.

The author very clearly observed the migratory tendency of goblet cell in *C. carpio*. This is due to the fact that they are produced from two sources : first by the transformation of marginal supporting cells and second by the muciferous basal cells. In the latter case the basal cell along with its transformation into the goblet cell undergoes cyclic movement from basal to the supporting zone. This brought the goblet cell, originated from basal cell, to the free surface where mucous is discharged.

In *C. carpio* the proximal intervening region of the lamella is pooled with muciferous basal cells, which undergoes a process of

transformation into the goblet cells. Whole of the olfactory epithelium of *C. carpio* can be seen with different sizes of goblet cells, in the way of their migration from basal to supporting zone. The tremendous tendency of production of goblet cells from muciferous basal cells for the first time observed by present author in *C. carpio*.

In *C. carpio* marginal goblet cells are resulted due to the continuous transformation of supporting cells into these cell types (goblet cells), therefore, whole of the marginal surface of lamellae is seen occupied by the theca of goblet cell except few original or transitional supporting cells. This is in agreement with the findings of Moe(1955) who described the goblet cell as the modified columnar supporting cells. The transitional stages of columnar supporting cells can be easily seen in the olfactory epithelium of *C. carpio* and *B. bagarius*.

In *B. bagarius* muciferous basal cells are not seen and goblet cells are produced by the nonciliated supporting cells of nonsensory region. They are also produced by the cuboidal supporting cells of posterior lamellae in *B. bagarius*.

In *T. mossambica* the olfactory mucosa is scantily supplied with the goblet cells but they are richly present in the accessory nasal sacs. The author is of the opinion that the rich supply of goblet cells in the accessory nasal sacs in *T. mossambica* is for smoothening the water supply and entangling the foreign material, permitting clear water to the sensory surface. This is in agreement with the findings of Rosen and Conford (1971).

The present author has attempted to classify the goblet cells under present investigation into two categories : (i) Stationary goblet

cells produced by the marginal supporting cells (2) Migratory goblet cells produced by the muciferous basal cells which undergo a course of migration from basal to supporting zone. In the former category, the theca is ovoid cup shaped and opens directly from the free surface of the olfactory epithelium and can be named as "Mega goblet cells", While later with rounded and comparatively smaller theca, may be termed as "Micro goblet cells".

C. carpio shows a tremendous capacity of transforming basal cells into the goblet cells which may be grouped and fused at variable depths in the olfactory epithelium. Due to the grouping and fusion of migratory goblet cells in large number, it causes the formation of complex structure which may burst from the general surface of the olfactory epithelium. This results in the formation of crypts of variable shapes where large number of receptor cells can be accommodated. In this way the area of sensory surface in the olfactory epithelium of *C. carpio* is greatly increased. The crypts along with the sensory elements appear like a well formed " olfactory crypt" which are found embedded at different depths in the olfactory epithelium.

The migration of goblet cells and their subsequent increase in the size of theca in the middle of olfactory epithelium, cause the displacement of basal cells which are forced to flow in any direction, resulting in microformations such as hillock elevation, straight projection, bifurcation and trifurcation. The enlarged theca of marginal goblet cells and its grouping with other migratory goblet cells cause the interruption of the olfactory epithelium in *C. carpio*

The basal cells are subjected to pressure of enlarged theca, which forces their rapid morphogenesis and flow to any direction

leading to a number of microformations on the general surface of the lamellae in *C. carpio*.

The grouping of goblet cells, formation of crypts and differentiation of goblet cells in mega and micro forms contribute to the unique finding of the present research work. Bertmar (1972) denied the possibility of grouping of goblet cells in the olfactory epithelium of fishes.

Bloom and Fawcett (1978) quoted that the secretion of mucous proceed more or less continuously and the life span of mucous secreting goblet cells is only two to four days in the intestinal mucosa. Although goblet cell normally passes through one long secretory cycle but they may be made to expel all of their secretion at once.

Similar secretory nature of the goblet cells is found in *C. carpio* and *B. bagarius*. After discharging the mucous, the goblet cells are supposed to dead and theca forms empty space where flow of basal cells may be possible. But sometimes number of empty goblet cells meet at a point and allow the detachment of the part of the lamella or "Cell ball" in *B. bagarius*. The distal ends of lamellae are seen continuously discharging the cell balls which may be due to the meeting of empty theca of goblet cells and subsequent narrowing of underneath region of detached portion. The detachment of terminal ends of the lamellae is also noticed in *C. carpio*.

In the process of budding in *B. bagarius*, the theca of goblet cells, after discharging the mucous provide a way for the flow of basal cells, which aggregate at distal end of the mother lamellae in the form of a bud. The further activity of goblet cell at the junction of bud and mother lamellae causes its (bud) detachment. The attachment of bud

on the recipient lamella is based on the morphogenetic activity of basal cells, with the enlargement of goblet cells and their flow in the way formed by the empty theca. The basal cells cause the elongation of bud and empty theca of goblet cells creates a place for the meeting of bud and recipient lamella which allow the fusion of each other. The cell balls and bud are mainly constituted of basal cells and goblet cells.

In *C. carpio*, bifurcation and trifurcation of the lamella is observed which are formed due to empty spaces created by the death of goblet cells after the discharge of mucous. The empty theca of these goblet cells exert pressure on underlying basal zone which makes forcible flow of basal cells in the direction of bifurcation and trifurcation for accommodating the bulk of basal cells generated by routing mitotic division. During this process of morphogenetic activity, crypts of different shapes and sizes and other surface specifications occurs due to the displacement of basal cells.

The association of goblet cell with the aquatic mode of life can be traced out because they are present in the fishes (Whitaker, 1970), amphibians (Farquhar and Pallade, 1965) and aquatic snakes (Banerjee and Mittal, 1978). Their presence in aquatic form is to minimise the friction between the body and water and thus increases the mobility of the animal. The mucous secretion also allow the smooth flow of water into the olfactory chamber and protects the sensory epithelium with direct effect of water friction in fishes.

The present author is of the opinion that goblet cells are certainly related with the increase in the surface of olfactory sensation and help in removing the debris from the olfactory surface by

entangling them in mucous secretion. The debris is ultimately removed outside by forcibly passing of water current in any direction.

Devitsyna (1972) studied two marine species *Gadus morhua*, *Elaginus novaga* and freshwater species *Lota lota* and concluded that, goblet cells can be assumed in some way to promote the olfactory active substances in the salt water. The author is of the view that goblet cells cannot be the only distinctive feature of marine fishes, as they are also observed in fresh water forms. The presence of goblet cell in the olfactory epithelium of *C. carpio*, *B. bagarius* and *T. mossambica* therefore contradicts the idea of Devitsyna (1972).

Basal Cells :

In all the vertebrates including fishes, the basal cells occupy proximal position just above the basement membrane (Allison, 1953; Graziadei, 1965; Andres, 1966; Wilson and Westerman, 1967; Gemne and Doving, 1967; Singh, 1972; Bertmar, 1972; Ojha and Kapoor, 1973; Kapoor and Ojha, 1974; Hara, 1975; Zeiske *et al.*, 1976; Bronshtein, 1976; Yamamota and Ueda, 1977; Rahmani and Khan, 1980; Moran *et al.*, (1992); Hansen and Zeiske, 1993; Byrd and Brunjes, 1995; Fishelson, 1995; Fishelson, 1995; and Eastman and Lannoo, 2001, 2003, 2004) These cells are undifferentiated and give rise to supporting cells (Schaeffer, 1932; Cordier, 1964; Ojha and Kapoor, 1973) or to the receptor cells (Andres, 1966; Thornhill, 1970; Graziadei and Metcalf, 1971) or both types of cells (Bertmar, 1972; Hara, 1975; Sharma, 1981; Singh and Singh, 1986; Whelan *et al.*, 1986; Yadav, 1989; Dubey, 1991; Sharma, 2002). According to Ross and Reith (1985) the basal cells characteristically lies above the basement membrane in variable number, performing morphogenesis

and transfer of nutritional contents from submucosal to mucosal region.

In the present study of *C. carpio*, *B. bagarius* and *T. mossambica*, the basal cells occupy the lower region forming clear cut basal zone just above the basement membrane. In *T. mossambica* basal cells are present in multilayer above the basement membrane in the middle part of the lamellae in an uniform way, but in extreme proximal and distal tip they are scattered irregularly and mixed with other transitional cellular contents

In *T. mossambica* the basal cells are abundantly present and show their frequent migration and accumulation to any direction in the olfactory mucosa, leading to the creation of elevations and deepening of different shapes and sizes.

The basal cells in *C. carpio* are abundantly present and show their frequent accumulation in the basal zone which lead to the replacement of damaged and worn out cellular component of the olfactory epithelium. The basal cells show a tendency of flow in any direction. In such region they accumulate in large numbers and undergo a process of transformation to other cellular component of olfactory epithelium. They act as cellular reservoirs and are greatly effected by the migratory goblet cells whose theca occupy larger area of olfactory epithelium. This causes displacement of the basal cells, leading to their flow in any direction of the formation of hillock elevation, straight projections, bifurcation and trifurcation on the general surface of the olfactory epithelium of *C. carpio*. The basal cells can also be observed in the supporting zone justifying their transformation into the supporting cells.

The present author for the first time identified the muciferous basal cells in olfactory epithelium of *C. carpio*. Such basal cells are richly accumulated in the proximal intervening regions on the either sides of raphe and show rich granulation. The muciferous basal cells undergo a cyclic migration from basal to supporting zone in the preparation of the formation of complete goblet cells, which discharge their mucous secretion at the free distal surface of olfactory epithelium in *C. carpio*. In *C. carpio* positively muciferous basal cells may group at any depth of the olfactory epithelium and form complex structure of crypts, which may be of variable shape and sizes, opening through the free surface of the lamella by broad and narrow opening. The differentiation of basal cells into primary supporting cells can be seen in *C. carpio*. Unexposed crypts are also observed in the olfactory epithelium of *C. carpio*, which lie embedded at different depths accommodating large number of primary neurons.

In *B. bagarius* the basal cells are distributed in two zones : first in sensory and supporting zone and second in indifferent epithelium of distal zone. In former they are scanty present in a single row but in later they form a thick and multilayered basal zone. They are also present in many layer in posterior lamellae below the cuboidal supporting cells. The basal cells show their flow into the terminally detached cell balls in middle lamellae and in posterior lamellae they flow to form bud. The bud and detached cell balls are richly supplied with the basal cells which may transform these fragments into the complete lamella or provide substitutional supply to the other part of the olfactory epithelium.

The basal cells are also observed in the accessory sacs of *B. bagarius* and *T. mossambica* which give rise to the formation of hillock elevations in the internal lining of the sac. The goblet cells and cuboidal supporting cells are also continuously replaced by the basal cells. In *C. carpio* and *B. bagarius* the raphe bears a clear basal zone which may be constituted of one or more layers of basal cells. The basal cells are also observed in the connective tissue of central core or submucosa of raphe in *C. carpio* and *B. bagarius*. The submucosa or central core of above fishes bear variable supply of basal cells, fibroblasts, lymphocytes and histocytes which are found impregnated among the connective tissue fibres.

Pigment Cells :

The epithelium pertaining to senses of hearing, olfaction, taste and touch is peculiarly supplied with pigment cells. The function of pigment cells is not fully known but it seems that they might be enhancing the smelling and hearing powers in the animal in some way or other (Allison, 1953). It is significant that albino animals in which the pigment cells are lacking, are particularly liable to poisoning (Allison, 1953). Malyukina *et al.* (1969) think that there exists a relationship between the intensity of colours of olfactory epithelium and the sensitivity of the organ of smell, darker the epithelium, higher the sensitivity. Hildebrand (1974) also favours the view that pigment may enhance olfaction in some unknown way.

In the present study the submucosa of raphe and lamellae is supplied with the pigment cells in *C. carpio* and *B. bogarius*. They are branched and entangled in the fibres of connective tissue. In case of *T.*

massombica pigmentation is not seen in submucosa or anywhere in the olfactory composition of fish.

Devitsyna (1972) on the basis of comparative study of three ganoid fishes concluded, that, pigmentation of olfactory plates is a characteristic feature of some fishes with a reduced olfactory function. *Novaga eleginus* bears pigment cells while the lamellae of *Lota lota* and *Godus morhua* are devoid of these cells.

The present author observed pigment cells in all macrosomatic species (*C. carpio* and *B. bagarius*) but are absent in microsmatic form (*T. mossambica*). Therefore, it can be concluded that presence of pigment cells is related with the increase in olfactory behaviour and is not with the reduction of olfactory function. The author contradicts the findings of Devitsyna (1972) and believes that the occurrence of pigment cells is the characteristics of a highly sensitive sensory organ.

Chapter-5

Histochemical Discussion

Histochemical Discussion

In the olfactory epithelium of higher vertebrates, both acid and alkaline phosphatase are reported to be widely distributed (Baradi and Bourne, 1953). The acid phosphatase activity in the olfactory epithelium of *Cyprinus carpio* is more pronounced and widespread than the alkaline phosphatase activity. The former wherever observed in cytoplasm, appeared to be localized in granules which could possibly be the lysosomes. In *T. mossambica*, the localization of acid phosphatase is also in granular form but in this species activities of both phosphatase seem to be moderate and of equal degree.

The primary and rod shaped receptors of *T. mossambica* and primary neurons and spindle shaped receptor cells of *C. carpio*, exhibit greater degree of alkaline phosphatase activity. In rod shaped receptor cells of *C. carpio* alkaline phosphatase activity is equally pronounced but localization is in granular form.

In teleost *Esox lucius*, Bronshtein (1962b) has reported that, non specific phosphatase are irregularly distributed in the olfactory cells.

In mammals and other vertebrates, the olfactory cells reportedly possess more alkaline phosphatase activity and little less acid phosphatase activity (Amicis and Zorzoli, 1957; Negri, 1957; Barbare, 1959; Bronshtein, 1960). Baradi and Bourne (1953) reported acid phosphatase activity in the olfactory cells of vertebrate but fail to demonstrate any enzyme in olfactory hairs (cilia). Bronshtein (1965) reported moderate alkaline phosphatase activity in these cilia. Gasser

(1956) found a distinct activity of phosphomonoesterases in the body of olfactory cell of vertebrates but obtained a moderate reaction for them in the axon and proximal part of the dendrite. The author observed alkaline phosphatase activity in the olfactory cilia, axon and terminal end of dendrite in both *T. mossambica* and *C. carpio*.

The ciliated supporting cells of the olfactory epithelium of *C. carpio* and *T. mossambica* have been reported to show negative alkaline phosphatase activity. The alkaline phosphatase activity in the cuboidal nonciliated supporting cell of *T. mossambica* is of very feeble degree. This activity may also be detected in nonciliated supporting cell of *C. carpio* but, in a very non recordable way.

According to Jinnin (1965) the supporting cells of rabbit are rich in acid phosphatase, while, on the other hand Baradi and Bourne (1953) reported only small quantity of acid phosphatase in the mammalian supporting cells. Lysosome in supporting cell of mammal have been described by Balboni (1965), Andres (1966). Bronshtein (1965) concluded that the activity of enzyme and other biologically active substances in the supporting cells is generally low and this indicates the primary role of receptor cells in the general metabolism of the olfactory mucosa.

The basal cells of the olfactory mucosa in mammal are rich in both acid and alkaline phosphatase (Baradi and Bourne, 1953; Jinnin, 1965; Shantha and Nakajima, 1970). In human olfactory mucosa these cells are reported to be largely phosphatase negative. Baradi and Bourne, 1953; Kleerekoper, 1969; Jinnin 1965 reported much alkaline phosphatase activity in basal cell of the rabbit.

Lysosomes have been described in these cells of mammals by Balbone (1965) and Andres (1966).

In *C. carpio* basal cells are rich in both acid and alkaline phosphatase but the activity of alkaline factory is very high. In *T. mossambica* the acid and alkaline phosphatase activity is rich in basal cell but the cells which are in active mitotic division phase reveal rich concentration of acid phosphatase as compared to other cells. The author thus conclude, that basal cell exhibit a distinct activity of acid and alkaline phosphates in both the species.

The observation of prominent localization of acid and alkaline phosphatase in the basal cell of *C. carpio* indicate that these cells are under the influence of heavy metobolic activity leading to creation of microformation on lamellar surface and supplementing the requirement of other cellular components for the olfactory mucosa. In *T. mossambica* although acid and alkaline phsophatase is clearly localized but not upto that degree as in C. carpio. This fact can be demonstrated in C. carpio where the basal cells of *C. carpio* is under the process of tremendous morphogenetic activity.

The basal cells in *C. carpio* are seen accumulated above the basement membrane in a regular fashion, which is supposed as their preparation in the direction of giving rise to other cellular forms of mucosa or helping in micro formation or in repair work under the condition of injuries and infections. High alkaline phosphatase activity in basal cell of this fish suggest that this might be for availing all the physiological requirement to the basal cells for these specific functions.

The mucous secretory goblet cell in the olfactory epithelium of *C. carpio* show acid phosphatase activity along their cell and nuclear membrane. No enzyme activity could be demonstrated in cytoplasm and their mucous secretion in goblet cell. Although *T. mossambica* shows very little mucus secretory activity, as goblet cells are restricted to the extreme distal tip but the moderate localization of acid phosphatase in the outline of the theca of the cell can be observed. Baradi and Bourne (1953), Jinnin (1965) found an intense reaction of acid phosphatase in the Bowman's gland of higher vertebrate while Shanta and Nakagima (1970) reported that Bowman's gland is rich in both acid and alkaline phosphatase. Thus, author could reach to a conclusion that Bowman's gland of mammals and mucous secretory cells of *C. carpio* and *T. mossambica* are discharging analogous function i.e. different in origin but similar in function.

The role of enzyme in olfaction is imperfectly understood. Kistiakowsky (1950) and Baradi and Bourne (1951, 1953) claim that excitation of olfactory receptors occur as a result of differential inhibition of enzyme activity in them by odoriferous substances. Ottoson (1963) pointed out that olfactory hair seem to contain little enzyme activity.

Brettschneider (1958) and Bronshtein (1960, 1962a, 1965) reported that a high activity of oxidative enzyme occurs in the mitochondria present in the distal portion of dendrite of olfactory receptor cell and in its vesicle. It is known that ATP is formed as a result of oxidative phosphorylation while inorganic phosphate required for the formation of ATP is provided by dephospholysis brought about

by acid alkaline phosphatase. The significance of alkaline phosphatase activity in the olfactory vesicle and in the distal part of dendrite could possibly be evaluated in above reference. Bronshtein (1965), even reported a moderate alkaline phosphatase activity in the olfactory cilia and claim the participation of ATP in their movement. It can perhaps be correctly argued that occurrence of alkaline phosphatase activity at the receptor sites in the olfactory epithelium may be concerned with transfer of energy from the odour molecules to these receptor sites and that the movement of olfactory cilia, in all probability, facilitate the process.

The secretion of goblet cell in the olfactory epithelium of *C. carpio* and *T. mossambica* is similar to the secretion of Bowman's gland in terrestrial vertebrates and is rich in mucopolysaccharides and also contain a good amount of glycogen (Amicis and Zarzoli, 1957; Bronshtein, 1960, 1962b; Duvegan and Jerebtzoff, 1967). The occurrence of lipid is in high concentration in *T. mossambica*. and *C. carpio* in their distal limb of supporting cell and in moderate concentration along with in primary neurons. The basal cells, secondary neurons and cilia of supporting cell exhibit relatively poor content of lipids. The lipid and glycogen content in *T. mossambica* are exactly localized similar to *C. carpio* but degree of concentration varies which can be seen from the table listed in observation chapter.

In mammals olfactory cells are reported to be rich in lipids (Amicis and Zorzoli, 1957). The primary and secondary neurons which establish synaptic contact exhibit high concentration of phospholipid. In contrast to it, the primary neurons of *C. carpio* exhibit slightly low

degree of localization of lipid. The lipids are highly concentrated in rod shaped receptor and spindle shaped receptor in *C. carpio*.

It is well known that lipid play an important role in the structure and function of cell membrane. The nature of the chemical reaction initiated in the olfactory receptor by odour molecule is not known. The author is of the opinion that there is the possibility of lipid solubility of the odorous substances and is one of the key factor in the whole chain of process of reception of olfactory sense.

In aquatic animals odorous substances pass on to the aqueous liquid surface of the olfactory membrane. The olfactory cilia is supposed to be the actual receptor site for odour molecule and their lipid nature is helpful in the sensation.

Onawaga (1957a, 1957b) demonstrated that the sensitivity of olfactory stimulation is directly proportional to the pigmentation of olfactory mucosa while Briggs and Demcan (1961, 1962) reported that these pigment cells in olfactory epithelium are receptors of energy from odour molecule. Mouton and Beidler (1967) and Kleerekoper (1968) gave the evidence that the pigment of olfactory epithelium in odour perception is not surely involved. The heavy pigmentation through the raphe of *C. carpio*, demonstrate its efficient way of olfactory reception.

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